

"POINT OF CARE DIAGNOSTIC METHODS"

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims priority from Australian Provisional Patent Application No. 2023900818 filed on 23 March 2023, the contents of which are
5 incorporated herein by reference in their entirety.

TECHNICAL FIELD

The present disclosure relates generally to voltammetric methods of biosensing nucleic acid analytes in complex biological samples and/or the use of such methods in diagnostic applications. More particularly, the disclosure relates to diagnostic methods
10 which use a voltammetric biosensing device comprising a porous substrate comprising a plurality of channels, wherein at least a portion of the plurality of channels comprise moieties e.g., capture probes, capable of binding with one or more nucleic acid analytes in complex biological samples. The one or more nucleic acid analytes may be biomarkers associated with an injury, condition or disease state, for example a brain injury, such as
15 a traumatic brain injury. The methods of the disclosure may optionally determine a concentration of the one or more nucleic acid analytes in the complex biological sample.

BACKGROUND

There is a lack of suitable diagnostic tools that can be adapted and deployed in a facile manner to assist in the detection of one or more conditions in a subject within an
20 appropriate timeframe. Early detection of a condition may be important to determine appropriate treatment options (and reduce the chance of complications). Hence, producing diagnostic tools which can be adapted to detect appropriate biomarkers associated with a disease state or injury in a timely manner is important.

An example of an injury that can lead to significant complications for a subject is
25 a traumatic brain injury (TBI), which may be an injury to the brain caused by an external force. TBI can be classified based on severity, ranging from mild to severe. TBI represents a major cause of death and disability worldwide. TBI can result in physical, cognitive, social, emotional and behavioural symptoms, and outcomes can range from complete recovery to permanent disability or death.

There is a lack of suitable diagnostic technologies that allow the accurate detection of TBI, and are able to determine the severity of TBI, within a suitably short timeframe. Diagnostic technologies that have been explored in the context of TBI have ranged from imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) to the detection and quantification of molecular markers ranging from proteins to miRNAs, but these approaches require time and resources, and there are difficulties in accessing appropriate instruments and devices in a timely and cost effective manner.

Among the diagnostic technologies available for TBI, the discovery of miRNA biomarkers associated with TBI, such as those described in PCT/US2015/051518, has shown particular promise. However, there remains a need for diagnostic tools that can rapidly and sensitively detect miRNAs (or other nucleic acid biomarkers for that matter), in complex biological samples in a point of care setting and within an appropriate timeframe.

Any discussion of documents, acts, materials, devices, articles, or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each of the appended claims.

SUMMARY

Disclosed herein are voltammetric methods of biosensing nucleic acid analytes in complex biological samples and the use of such methods in diagnostic applications. More particularly, the disclosure relates to diagnostic methods which use a voltammetric biosensing device comprising a porous substrate having a plurality of channels, a portion of which comprise capture probes capable of binding with one or more nucleic acid analytes and configured to detect one or more nucleic acid biomarkers in a complex biological sample. In some examples, the nucleic acid analytes which may be detected using the method of the disclosure are indicative of traumatic brain injury (TBI). The methods of the disclosure may therefore be used to diagnose TBI based on the detection of one or more nucleic acid analytes or biomarkers associated with TBI (e.g., miRNAs), in complex biological samples such as blood and blood fractions, and optionally, potentially at the same time, determine the severity of TBI based on biomarker concentration and/or profile. However, it will be appreciated that the method of the disclosure may be used for biosensing other nucleic acid analytes or biomarkers present in complex biological samples in a range of other diagnostic applications.

An aim of the present disclosure is, *inter alia*, to assist the clinical decision making process and ultimately contribute to significantly improved management of conditions of TBIs and, where possible, improve clinical and operational outcomes, by enabling more rapid detection of biomarkers associated with TBI. In working towards this goal, the inventors developed a diagnostic assay for TBI which is based on nucleic acid-based biomarkers (i.e., microRNA), and which is capable of being performed in a 'near-patient' setting, such as in a field hospital or on a sideline. Whilst the integration of certain diagnostic technologies into benchtop and handheld 'point-of-care' devices has certainly gone some way to enabling patient assessment and/or diagnosis in 'near-patient' settings for a range of conditions (e.g., lateral flow antibody-based diagnostic tests), for diagnostic assays based on nucleic acid based biomarkers (such as TBI), the need for sample pre-treatment or preparation to transform complex biological samples from their collected form to a form suitable for analysis has presented a barrier to the adoption of point of care platforms. In this regard, leading up the present disclosure, diagnostic assays and methods configured to detect nucleic acid analytes were poorly adapted to be performed on complex biological samples directly or with minimal sample preparation.

Herein, the method of detecting nucleic acid analytes, such as nucleic acid biomarkers, in complex biological samples may utilise a device comprising manufactured porous substrate, for example a porous substrate comprising silicon (pSi). The porous substrate may comprise channels (for example on the micron and/or nanometre scale), with controlled dimensions in relation to depth and/or cross section. The porous substrate may be stabilised (for example to reduce degradation, chemical reactions and/or improve electrochemical stability, such as via thermal (hydro)carbonisation), followed by the introduction of capture probes, or precursors to the capture probes, for example the introduction of the functional groups that can be modified to become capture probes or act as anchor points on at least one surface of the porous substrate, for example within at least a portion of the plurality of channels, to attach and immobilise one or more capture probes. The stabilisation may involve exposing the porous substrate to an elevated temperature for a period of time.

When performing the method of the disclosure, one or more nucleic acid analytes, for example DNA, mRNA or miRNA analytes, may be bound to one or more capture probes disposed in at least a portion of the plurality of channels of the porous substrate. The one or more nucleic acid analytes may be detected by electrochemical measurements, using appropriate means, for example a potentiostat. Calibration curves,

graphs and data may be obtained by obtaining electrochemical measurements using compositions with known concentrations of one or more analytes.

The porous substrate may form part of an electrochemical sensor, wherein the binding of one or more analytes to immobilised complementary capture probes, within at least a portion of the plurality of channels, induces at least a partial blockage, which in turn hinders the diffusion of a redox species, for example $[\text{Fe}(\text{CN})_6]^{3-/4-}$, into an electrochemically active layer (for example an electrochemical transducer), thereby resulting in a decrease in the intensity of peak current, which can be detected and quantified. For sensors deployed and/or exposed to biological samples, the signal from any channel blockage can also be influenced by biofouling mechanisms, for example where foulants attach to a portion of an electrode (for example an electrode derived from or based on a porous substrate as described herein), via non-specific adsorption. The foulants can potentially reduce the area of electrode surface that is available to one or more analytes, causing a decrease, for example, of a differential pulse voltammetry signal. Herein, the control and selection of the dimensions of the plurality of channels can negate or reduce problems associated with biofouling, thereby improving the ability to detect nucleic acid analytes in complex biological samples. Without being bound by any one theory, this may, at least in part, be due to the dimensions of the channel being on a scale such that proteins and/or other potential biomolecules that give rise to fouling (such as those that may be present in complex biological samples comprising the analytes of interest), cannot enter or progress through a channel, whilst the analytes of interest are on a scale that means they are sufficiently small and mobile to reach capture probes disposed within at least a portion of the plurality of channels. Thus, an advantage of the method of the disclosure may be the ability to detect the presence of one or more nucleic acid analytes in a biologically complex media directly with minimal or reduced biofouling. The ability to be able to detect nucleic acid analytes in complex biological media (e.g., blood), using the method of the disclosure may reduce the need to process samples prior to testing and/or minimise human handling which can lead to contamination, degradation and/or error. As discussed above, the ability to perform a diagnosis on a complex biological sample, such as blood or serum, may also permit tests to be performed in the field, thereby reducing the timeframe in which a diagnosis can be made. This may also reduce the need to transport one or more samples to a separate environment/facility for testing and/or analysis, potentially reducing the time for obtaining any results, and also potentially allowing a diagnosis to be determined (and acted upon), within a shorter timeframe, thereby reducing detrimental effects on a subject.

It would also be an advantage if measurements could be obtained from a broad range of samples (potentially derived from differing sources), and provided in different forms. This would allow for a flexibility with sample testing (for example a sample in a particular form and/or purity and/or concentration is not needed). The type or form of the sample could include liquid samples obtained from a subject, for example samples in the form of blood and/or serum, which may be used directly, or following dilution in a suitable solvent (such as buffered solution or saline). In this regard, minimal or no sample preparation may be required.

Another possible advantage of the methods disclosed herein may not only be detecting the presence of one or more nucleic acids analytes in complex biological samples to determine the existence of a medical condition, (such as TBI), but also quantifying the amount of the one or more nucleic acids analytes to potentially determine the severity of said condition (for example by using calibration curves developed using appropriate standards, and identifying thresholds associated with different stages of severity). This may assist with triage matters and/or determining the most appropriate course of treatment for a subject. This determination could potentially occur within a short timeframe, allowing for quick assessment of a situation and allowing appropriate treatment steps to occur with reduced and/or minimal delays.

Accordingly, in a first aspect, the present disclosure provides a method of detecting at least one biomarker, for example at least one nucleic acid analyte, in a complex biological sample, the method comprising:

- (i) introducing the complex biological sample to an electrochemical biosensor comprising a porous substrate comprising a plurality of channels, wherein:
 - at least a portion of the plurality of channels has a mean depth of about 1.0 μm to about 6.0 μm ; and/or
 - at least a portion of the plurality of channels has a mean diameter or cross section in a range of about 15 nm to about 40 nm,

wherein one or more capture probes capable of binding specifically to at least one biomarker, for example at least one nucleic acid analyte, to form a probe-analyte complex are disposed on at least one surface of at least a portion of the plurality of channels; and

- (ii) determining the presence or absence of the at least one biomarker, for example the at least one nucleic acid analyte, in the sample, optionally based on measurements of peak current obtained from differential pulse voltammograms prior to and after introducing the complex biological sample to the electrochemical biosensor, wherein detection of a change in the peak current after

introduction of the complex biological sample to the electrochemical biosensor indicates the presence of the at least one biomarker, for example the at least one nucleic acid analyte, in the complex biological sample and detection of no change in the peak current after introduction of the complex biological sample to the electrochemical biosensor indicates that the at least one biomarker, for example the at least one nucleic acid analyte, is not present in the complex biological sample above a threshold level.

In one example, the capture probe is capable of binding specifically to at least one nucleic acid analyte to form a probe-analyte complex, wherein the presence of the probe-analyte complex within a channel causes at least a partial steric and/or charge blockage to the channel as compared to the channel in the absence of the probe-analyte complex.

In one example, at least a portion of the plurality of channels has a mean depth of about 1.0 μm to about 6.0 μm , and at least a portion of the plurality of channels has a mean diameter or cross section in a range of about 15 nm to about 40 nm. For example at least a portion of the plurality of channels has a mean depth of about 1.0 μm to about 6.0 μm , and a mean diameter or cross section in a range of about 15 nm to about 40 nm.

In one example, at least 10% of the plurality of channels have a mean depth in a range of about 1.0 μm to about 6.0 μm . For example, at least 15% of the plurality of channels may have a mean depth in a range of about 1.0 μm to about 6.0 μm . For example, at least 20% of the plurality of channels may have a mean depth in a range of about 1.0 μm to about 6.0 μm . For example, at least 25% of the plurality of channels may have a mean depth in a range of about 1.0 μm to about 6.0 μm . For example, at least 30% of the plurality of channels may have a mean depth in a range of about 1.0 μm to about 6.0 μm . For example, at least 35% of the plurality of channels may have a mean depth in a range of about 1.0 μm to about 6.0 μm . For example, at least 40% of the plurality of channels may have a mean depth in a range of about 1.0 μm to about 6.0 μm . For example, at least 45% of the plurality of channels may have a mean depth in a range of about 1.0 μm to about 6.0 μm . For example, at least 50% of the plurality of channels may have a mean depth in a range of about 1.0 μm to about 6.0 μm .

Alternatively or in addition to the at least 10% of the plurality of channels having a mean depth in a range of about 1.0 μm to about 6.0 μm as described hereinabove, at least 10% of the plurality of channels may have a mean diameter or cross section in a range of about 15 nm to about 40 nm. For example, at least 15% of the plurality of channels may have a mean diameter or cross section in a range of about 15 nm to about 40 nm. For example, at least 20% of the plurality of channels may have a mean diameter

or cross section in a range of about 15 nm to about 40 nm. For example, at least 25% of the plurality of channels may have a mean diameter or cross section in a range of about 15 nm to about 40 nm. For example, at least 30% of the plurality of channels may have a mean diameter or cross section in a range of about 15 nm to about 40 nm. For example, at least 35% of the plurality of channels may have a mean diameter or cross section in a range of about 15 nm to about 40 nm. For example, at least 40% of the plurality of channels may have a mean diameter or cross section in a range of about 15 nm to about 40 nm. For example, at least 45% of the plurality of channels may have a mean diameter or cross section in a range of about 15 nm to about 40 nm. For example, at least 50% of the plurality of channels may have a mean diameter or cross section in a range of about 15 nm to about 40 nm.

In some examples, the porous substrate comprises, consists essentially of, or consists of, conducting or semi-conducting material. In some examples, the porous substrate comprises, consists essentially of, or consists of, a material selected from the group consisting of: platinum, gold, silver, cobalt, gallium, indium, tin, vanadium, zirconium, copper, aluminium, iridium, palladium, rhodium, silicon, zinc, iron, steel, brass, carbon, graphite, alloys, oxides, or metallic compounds of these elements, and mixtures thereof. For example, the porous substrate may comprise, consist essentially of or consist of silicon.

In some examples, a layer comprising, consisting essentially thereof, or consisting of carbon may be disposed on at least a portion of the porous substrate.

In one embodiment, the porous substrate comprises silicon, wherein the silicon comprises at least one layer which has been modified via at least one of a heat and/or chemical treatment.

In another embodiment, the porous substrate, optionally comprising, consisting essentially or consisting of silicon, comprises, consists essentially of or consists of a single layer comprising one or more of:

- one or more capture probes capable of binding specifically to at least one biomarker, for example at least one nucleic acid analyte, to form a probe-analyte complex; and/or
- one or more functional groups capable of reacting with an amine group; and/or
- one or more functional groups capable of forming a covalent bond with a complementary functional groups on one or more capture

probes that are capable of binding specifically to at least one biomarker, for example at least one nucleic acid analyte, to form a probe-analyte complex; and/or

- one or more carboxylic acid groups.

5 In another embodiment, the porous substrate, optionally comprising, consisting essentially or consisting of silicon, comprises a layer comprising one or more of:

- one or more capture probes capable of binding specifically to at least one biomarker, for example at least one nucleic acid analyte, to form a probe-analyte complex; and/or
- 10 ▪ one or more functional groups capable of reacting with an amine group; and/or
- one or more functional groups capable of forming a covalent bond with a complementary functional groups on one or more capture probes that are capable of binding specifically to at least one biomarker, for example at least one nucleic acid analyte, to form a probe-analyte complex; and/or
- 15 ▪ one or more carboxylic acid groups.

In another embodiment, the porous substrate, optionally comprising, consisting essentially or consisting of silicon, comprises at least one layer comprising one or more of:

- 20 ▪ one or more capture probes capable of binding specifically to at least one biomarker, for example at least one nucleic acid analyte, to form a probe-analyte complex; and/or
- one or more functional groups capable of reacting with an amine group; and/or
- 25 ▪ one or more functional groups capable of forming a covalent bond with a complementary functional groups on one or more capture probes that are capable of binding specifically to at least one biomarker, for example at least one nucleic acid analyte, to form a probe-analyte complex; and/or
- 30 ▪ one or more carboxylic acid groups.

In another embodiment, the porous substrate, optionally comprising, consisting essentially or consisting of silicon, does not comprise or consist essentially of or consist of a plurality of layers comprising one or more of:

- one or more capture probes capable of binding specifically to at least one biomarker, for example at least one nucleic acid analyte, to form a probe-analyte complex; and/or
- 5 ▪ one or more functional groups capable of reacting with an amine group; and/or
- 10 ▪ one or more functional groups capable of forming a covalent bond with a complementary functional groups on one or more capture probes that are capable of binding specifically to at least one biomarker, for example at least one nucleic acid analyte, to form a probe-analyte complex; and/or
- 15 ▪ one or more carboxylic acid groups.

In some of the examples disclosed herein, the one or more capture probes are capable of binding specifically to one or more a nucleic acid analytes. In some examples, the porous substrate comprises a plurality of capture probes, each capable of binding
15 specifically to one or more a nucleic acid analytes.

The at least one nucleic acid analyte may be a biomarker. For example, the nucleic acid may be selected from the group consisting of: a DNA analyte, a messenger RNA (mRNA) analyte, ribosomal RNA (rRNA) analyte, a transfer RNA (tRNA) analyte, a microRNA (miRNA) analyte, a short interfering RNA (siRNA) analyte, plasmid DNA
20 (pDNA) and mixtures thereof. In one example, the nucleic acid analyte is miRNA.

The capture probe may be selected to suit the nucleic acid analyte to be detected. For example, one or more of the at least one capture probe may be a DNA capture probe or a peptide nucleic acid probe (PNA) probe. In one example, at least one capture probe is a DNA capture probe. In one example, at least one capture probe is a PNA probe. In
25 any of the examples disclosed herein, the capture probe may have modified chemistry, such as to improve stability, improve binding affinity and/or reduce off target effects of the capture probe. Modified chemistry for DNA and PNA probes is known in the art and contemplated herein.

The method may be used for detecting nucleic acid analytes or biomarkers associated with conditions, diseases or injuries and making a diagnosis regarding same. Accordingly, the at least one nucleic acid analyte may be associated with a condition, disease state or injury. In one particular example, the at least one nucleic acid analyte is associated with a brain injury, optionally a traumatic brain injury (TBI), and the method is for diagnosing a brain injury (e.g., TBI). However, other medical indications are
30 described and contemplated herein.

In one particular example, the method comprises detecting the presence or absence of one or more miRNA associated with TBI in a complex biological sample (e.g., blood, a blood fraction or a diluted blood solution). For example, the one or more miRNA may be selected from the group consisting of miR-142-3p, miR-196b-5p, let-7f-5p, miR-150-5p, miR-196b-5p and combination thereof.

In some examples, the method comprises determining the severity of a condition, disease state or injury by determining a concentration of at least one biomarker, for example at least one nucleic acid analyte, in a sample. For example, the disease state or injury may be a brain injury, such as a TBI, and determining a concentration of at least one nucleic acid analyte associated with the brain injury (e.g., TBI), is used to determine severity of the brain injury in the subject from which the sample was obtained. In accordance with this example, the nucleic acid analyte will be associated with brain injury e.g., TBI, and present at a concentration which correlates with severity of the injury.

As described herein, the method of the disclosure may be performed on complex biological sample or media. In some instances herein, the terms and phrases “complex biological sample” “sample” and “media” may be used interchangeably. In some examples, the sample may be a liquid sample. In one example, the liquid sample is a bodily fluid. Bodily fluids may include but are not limited to one or more of: whole blood, fractionated blood, serum, plasma, urine, saliva, sweat, spinal fluid, mucus (e.g., nasal drainage and phlegm), amniotic fluid, aqueous humor, vitreous humor, breast milk, cerebrospinal fluid (CSF), pericardial fluid, peritoneal fluid, pleural fluid, pus, rheum, saliva, sebum (skin oil), semen, sputum, sweat, synovial fluid, tears, vomit, urine and exhaled condensate. In other examples, the liquid sample is a cell lysate, cell culture medium, and mixtures thereof. In certain examples, the method may be used to detect at least one nucleic acid analyte in the complex biological sample directly. In other examples, the complex biological sample may be subjected to one or more processes (e.g., dilution, lysis, fractionation), prior to detection with the porous substrate or device comprising same. In some of the examples disclosed herein describing complex biological samples, one or more of the samples may be obtained from a mammalian subject, such as a human. In one example, the sample is obtained from a subject, optionally a human subject, and optionally comprising or consisting of: whole blood, serum, plasma, urine, saliva, sweat, spinal fluid, sputum, cell lysate, and mixtures thereof.

In accordance with other examples in which the method of the disclosure is used to detect a nucleic acid analyte associated with an allergen or contaminant, the complex biological sample may be a biological or organic sample selected from, but not limited to: a soil sample, a water sample, a plant material, a food or beverage sample, an agricultural product, surface debris, a surface swab or any combination thereof. In some of the examples disclosed herein, the complex biological sample may be obtained in a liquid form or processed to a liquid form for contacting with the porous substrate.

In accordance with any of the examples disclosed herein, the method of the disclosure may be capable of detecting at least one nucleic acid analytes in a complex biological sample at a concentration of about 1 pM or less.

The electrochemical sensor may comprise one or more electrodes. For example, the electrochemical sensor may comprise:

- a working electrode comprising the porous substrate described herein;
- a reference electrode; and
- a counter electrode.

In one example, an insulating material is disposed on at least a portion of the electrodes.

In one example, the electrochemical sensor is capable of scanning a potential within a certain range and measure current. In one example, the method, the electrochemical sensor comprises a potentiostat.

In one example, a change in the peak current is normalised via the relationship of Equation (1):

$$\Delta I = (I_0 - I_n)/I_0 \quad (1)$$

where ΔI is a normalised current change, I_0 is a peak current value measured after incubation for a first period of time in a buffer, and I_n is the peak current value measured after incubation for a second period in a target solution at a given concentration, in comparison to a control sample.

In one example, the first period of time and/or the second period of time is independently about or less than about: 60 minutes, 50 minutes, 40 minutes, 30 minutes or 20 minutes. For example, the first period of time and/or the second period of time is independently about or less than about 60 minutes. For example, the first period of time and/or the second period of time is independently about or less than about 50 minutes. For example, the first period of time and/or the second period of time is independently

about or less than about 40 minutes. For example, the first period of time and/or the second period of time is independently about or less than about 30 minutes. For example, the first period of time and/or the second period of time is independently about or less than about 20 minutes. In some example, the first period of time and second period of time are the same. In other examples, the first period of time and second period of time are different.

In one example, the method comprises determining the concentration of the nucleic acid analyte detected in the complex biological sample based on a level of reduction in peak current intensity. In some examples, the method further comprises comparing the concentration of the at least one nucleic acid analyte with a calibration curve.

In one particular example, the complex biological sample is:

- from a human; and/or
- a liquid sample; and/or
- selected from the group consisting of: whole blood, serum, plasma, urine, saliva, sweat, spinal fluid, sputum, cell lysate, cell culture medium and mixtures thereof; and/or
- obtained from a subject suspected of having suffered a brain injury (e.g., a TBI).

Further, whilst diagnostic applications in humans are clearly contemplated, so too are diagnostic applications in non-human animals such as, but not limited to: livestock, companion animals, working animals and laboratory animals. Accordingly, the subject in which a condition, disease or injury is to be detected may be any subject. Therefore, the porous substrate can be tailored for the detection of one or more specific biomarkers relating to a particular species and/or condition of interest.

In one example, the at least one nucleic acid analyte is detected, and/or concentration quantified, within: about 60 minutes, about 50 minutes, about 40 minutes, about 30 minutes, about 20 minutes, about 15 minutes or about 10 minutes of introducing the sample to the electrochemical biosensor. For example, the nucleic acid analyte may be detected, and/or concentration quantified, within about 60 minutes of introducing the sample to the electrochemical biosensor. For example, the nucleic acid analyte may be detected, and/or concentration quantified, within about 50 minutes of introducing the sample to the electrochemical biosensor. For example, the nucleic acid analyte may be detected, and/or concentration quantified, within about 40 minutes of introducing the sample to the electrochemical biosensor. For example, the nucleic acid analyte may be detected, and/or concentration quantified, within about 30 minutes of introducing the

sample to the electrochemical biosensor. For example, the nucleic acid analyte may be detected, and/or concentration quantified, within about 20 minutes or less of introducing the sample to the electrochemical biosensor.

5 In a second aspect, the present disclosure provides a porous substrate when used in the method of the first aspect, said porous substrate comprising a plurality of channels, wherein:

- at least a portion of the plurality of channels has a mean depth of about 1.0 μm to about 6.0 μm ; and/or
- 10 - at least a portion of the plurality of channels has a mean diameter or cross section in a range of about 15 nm to about 40 nm,

wherein one or more capture probes capable of binding specifically to at least biomarker, for example at least one nucleic acid analyte, to form a probe-analyte complex are disposed on at least one surface of at least a portion of the plurality of channels.

15 In one example, the capture probe is capable of binding specifically to at least one nucleic acid analyte to form a probe-analyte complex, wherein the presence of the probe-analyte complex within a channel causes at least a partial steric and/or charge blockage to the channel as compared to the channel in the absence of the probe-analyte complex.

20 In one example, at least a portion of the plurality of channels has a mean depth of about 1.0 μm to about 6.0 μm , and at least a portion of the plurality of channels has a mean diameter or cross section in a range of about 15 nm to about 40 nm. For example at least a portion of the plurality of channels has a mean depth of about 1.0 μm to about 6.0 μm , and a mean diameter or cross section in a range of about 15 nm to about 40 nm.

25 In one example, at least 10% of the plurality of channels have a mean depth in a range of about 1.0 μm to about 6.0 μm . For example, at least 15% of the plurality of channels may have a mean depth in a range of about 1.0 μm to about 6.0 μm . For example, at least 20% of the plurality of channels may have a mean depth in a range of about 1.0 μm to about 6.0 μm . For example, at least 25% of the plurality of channels may have a mean depth in a range of about 1.0 μm to about 6.0 μm . For example, at least 30% of the plurality of channels may have a mean depth in a range of about 1.0 μm to about 6.0 μm . For example, at least 35% of the plurality of channels may have a mean depth in a range of about 1.0 μm to about 6.0 μm . For example, at least 40% of the plurality of channels may have a mean depth in a range of about 1.0 μm to about 6.0 μm . For example, at least 45% of the plurality of channels may have a mean depth in a range of about 1.0 μm to about 6.0 μm . For example, at least 50% of the plurality of channels may have a mean depth in a range of about 1.0 μm to about 6.0 μm .

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Alternatively or in addition to the at least 10% of the plurality of channels having a mean depth in a range of about 1.0 μm to about 6.0 μm as described hereinabove, at least 10% of the plurality of channels may have a mean diameter or cross section in a range of about 15 nm to about 40 nm. For example, at least 15% of the plurality of channels may have a mean diameter or cross section in a range of about 15 nm to about 40 nm. For example, at least 20% of the plurality of channels may have a mean diameter or cross section in a range of about 15 nm to about 40 nm. For example, at least 25% of the plurality of channels may have a mean diameter or cross section in a range of about 15 nm to about 40 nm. For example, at least 30% of the plurality of channels may have a mean diameter or cross section in a range of about 15 nm to about 40 nm. For example, at least 35% of the plurality of channels may have a mean diameter or cross section in a range of about 15 nm to about 40 nm. For example, at least 40% of the plurality of channels may have a mean diameter or cross section in a range of about 15 nm to about 40 nm. For example, at least 45% of the plurality of channels may have a mean diameter or cross section in a range of about 15 nm to about 40 nm. For example, at least 50% of the plurality of channels may have a mean diameter or cross section in a range of about 15 nm to about 40 nm.

In some examples, the porous substrate comprises, consists essentially of, or consists of, a conducting or semi-conducting material. In some examples, the porous substrate comprises, consists essentially of, or consists of, a material selected from the group consisting of: platinum, gold, silver, cobalt, gallium, indium, tin, vanadium, zirconium, copper, aluminium, iridium, palladium, rhodium, silicon, zinc, iron, steel, brass, carbon, graphite, alloys, oxides, or metallic compounds of these elements, and mixtures thereof. For example, the porous substrate may comprise, consist essentially of or consist of silicon.

In some examples, a layer comprising, consisting essentially thereof, or consisting of carbon may be disposed on at least a portion of the porous substrate.

In some of the examples disclosed herein, the one or more capture probes are capable of binding specifically to one or more a nucleic acid analytes. In some examples, the porous substrate comprises a plurality of capture probes, each capable of binding specifically to one or more a nucleic acid analytes.

The at least one nucleic acid analyte may be a biomarker. For example, the nucleic acid may be selected from the group consisting of: a DNA analyte, a messenger RNA (mRNA) analyte, ribosomal RNA (rRNA) analyte, a transfer RNA (tRNA) analyte, a

microRNA (miRNA) analyte, a short interfering RNA (siRNA) analyte, plasmid DNA (pDNA) and mixtures thereof. In one example, the nucleic acid analyte is miRNA.

The capture probe may be selected to suit the nucleic acid analyte to be detected. For example, one or more of the at least one capture probe may be a DNA capture probe or a peptide nucleic acid probe (PNA) probe. In one example, at least one capture probe is a DNA capture probe. In one example, at least one capture probe is a PNA probe. In any of the examples disclosed herein, the capture probe may have modified chemistry, such as to improve stability, improve binding affinity and/or reduce off target effects of the capture probe. Modified chemistry for DNA and PNA probes is known in the art and contemplated herein.

The present disclosure therefor provides the porous substrate as described herein when used to detect at least one nucleic acid analyte. Exemplary types of nucleic acid analyte are described herein and may be selected from the group consisting of: a DNA analyte, a messenger RNA (mRNA) analyte, ribosomal RNA (rRNA) analyte, a transfer RNA (tRNA) analyte, a microRNA (miRNA) analyte, a short interfering RNA (siRNA) analyte, plasmid DNA (pDNA) and mixtures thereof.

As described herein, it is contemplated that the porous substrate of the disclosure may be used for detecting nucleic acid analytes or biomarkers associated with conditions, diseases or injuries. Accordingly, the at least one nucleic acid analyte may be associated with condition, disease state or injury. In one particular example, the at least one nucleic acid analyte is associated with a brain injury, optionally a traumatic brain injury (TBI). However, other medical indications are described and contemplated herein.

In one particular example, the porous substrate comprises one or more capture probes capable of binding specifically to one or more miRNA associated with TBI in a complex biological sample (e.g., blood, a blood fraction or a diluted blood solution). For example, the one or more miRNA may be selected from the group consisting of miR-142-3p, miR-150-5p, miR-196b-5p, let-7f-5p, miR-196b-5p and combination thereof. The porous substrate of the disclosure may be used for detecting nucleic acid analytes in a range of samples and sample types. However, in one example, the sample is a liquid sample. Alternatively, the sample has been prepared or process as a liquid form. The liquid sample may comprise or consist of biological sample or media, for example a complex biological sample or media. In one example, the liquid sample is a bodily fluid. Bodily fluids may include but are not limited to one or more of: whole blood, fractioned blood, serum, plasma, urine, saliva, sweat, spinal fluid, mucus (e.g., nasal drainage and phlegm), amniotic fluid, aqueous humor, vitreous humor, breast milk, cerebrospinal fluid

(CSF), pericardial fluid, peritoneal fluid, pleural fluid, pus, rheum, saliva, sebum (skin oil), semen, sputum, sweat, synovial fluid, tears, vomit, urine and exhaled condensate.

In other examples, the liquid sample is a cell lysate, cell culture medium, and mixtures thereof. In certain examples, porous substrate may be used to detect at least one nucleic acid analyte in a liquid sample directly. In other examples, the liquid sample may be subjected to one or more processes (e.g., dilution, lysis, fractionation), prior to detection with the porous substrate or device comprising same. In some of the examples disclosed herein describing biological liquid samples, the sample may be obtained from a mammalian subject, such as a human. In one example, the sample is obtained from a subject, optionally a human subject, and optionally comprising or consisting of: whole blood, serum, plasma, urine, saliva, sweat, spinal fluid, sputum, cell lysate, and mixtures thereof.

In accordance with other examples in which the porous substrate of the disclosure is used to detect a nucleic acid analyte associated with an allergen or contaminant, the sample may be a biological or organic sample selected from, but not limited to: a soil sample, a water sample, a plant material, a food or beverage sample, an agricultural product, surface debris, a surface swab or any combination thereof. In some of the examples disclosed herein, the biological or organic sample may be obtained in a liquid form or processed so to a liquid form for use with the porous substrate.

In accordance with any of the examples disclosed herein, the porous substrate may be capable of detecting at least one nucleic acid analytes in a sample at a concentration of about 1 pM or less. When used to of detect at least one nucleic acid analyte in a sample, the porous substrate, electrode, electrochemical cell and/or device as described herein shall be capable of doing so within 20, 30, 40, 50 or 60 minutes of contacting the sample. In one example, the porous substrate, electrode, electrochemical cell and/or device as described herein is capable of detecting the at least one nucleic acid analyte in a sample within 20 minutes of contacting the sample. In one example, the porous substrate, electrode, electrochemical cell and/or device as described herein is capable of detecting the at least one nucleic acid analyte in a sample within 30 minutes of contacting the sample. In one example, the porous substrate, electrode, electrochemical cell and/or device as described herein is capable of detecting the at least one nucleic acid analyte in a sample within 40 minutes of contacting the sample. In one example, the porous substrate, electrode, electrochemical cell and/or device as described herein is capable of detecting the at least one nucleic acid analyte in a sample within 50 minutes of contacting the sample. In one example, the porous substrate, electrode, electrochemical cell and/or

device as described herein is capable of detecting the at least one nucleic acid analyte in a sample within 60 minutes of contacting the sample.

BRIEF DESCRIPTION OF DRAWINGS

Whilst it will be appreciated that a variety of embodiments disclosed herein may be utilised, described herein are a number of examples with reference to the following drawings:

Figure 1 depicts top-surface and cross-sectional SEM images of various thermally hydrocarbonised porous silicon (THCpSi) samples prepared in Example 1, under different conditions.

Figure 2 depicts water contact angle measurements of the pSi and THCpSi samples prepared in Example 1.

Figure 3 depicts FTIR spectra of freshly etched pSi, THCpSi, and thermally hydrosilylated THCpSi, prepared in Example 2.

Figure 4 depicts (a) cyclic voltammograms of freshly etched pSi, THCpSi, COOH-terminated THCpSi, and ssDNA-modified THCpSi electrodes prepared according to Example 3, along with (b) electrochemical impedance spectroscopy spectra of the THCpSi, COOH-terminated THCpSi and ssDNA-modified THCpSi electrodes.

Figure 5 depicts differential pulse voltammograms (DPVs) of the control and working electrodes fabricated by THCpSi with varied thickness according to Example 4, along with dosage response curves of the biosensors fabricated by THCpSi.

Figure 6 depicts differential pulse voltammograms (DPVs) of the control and working electrodes fabricated by THCpSi with varied channel sizes according to Example 4, along with dosage response curves of the biosensors fabricated by THCpSi.

Figure 7 depicts the sensing of target miRNA (miR-142-3p) using the optimised THCpSi-based nanochannel blockage biosensor, according to Example 5.

Figure 8 depicts differential pulse voltammograms (DPVs) of the biosensor upon incubations in clinic samples, according to Example 6.

Figure 9 depicts the calculated concentrations of miR-142-3p biomarker in three different clinic human serum samples.

Figure 10 depicts the screen printed porous silicon electrode assembly steps according to Example 7.

Figure 11 depicts insulator ink screen printing onto a silicon wafer according to Example 7.

Figure 12 depicts different stencils used for printing different electrode designs according to Example 7.

5 **Figure 13** depicts a new electrode design utilising a pSi working electrode, an Ag/AgCl reference electrode and a carbon-based counter electrode according to Example 7.

DETAILED DESCRIPTION

General Definitions

10 Unless specifically defined otherwise, all technical and scientific terms used herein shall be taken to have the same meaning as commonly understood by an individual skilled in a technical field (e.g., chemistry, biochemistry, engineering, physics and the like).

15 As used herein, the term “and/or”, e.g., “X and/or Y” shall be understood to mean either “X and Y” or “X or Y” and shall be taken to provide explicit support for both meanings or for either meaning, e.g., A and/or B includes the options i) A, ii) B or iii) A and B.

As used herein, the term about, unless stated to the contrary, refers to +/- 20%, or +/- 10%, or +/- 5%, of the designated value.

20 As used herein, the terms “a”, “an” and “the” include both singular and plural aspects, unless the context clearly indicates otherwise.

25 It is to be appreciated that certain features that are, for clarity, described herein in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features that are, for brevity, described in the context of a single embodiment, may also be provided separately or in any sub-combination.

30 Throughout the present specification, various aspects and components of the present disclosure can be presented in a range format. The range format is included for convenience and should not be interpreted as an inflexible limitation on the scope of the disclosure. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range, unless specifically indicated. For example, description of a range such as from 1 to 5 should be considered to have specifically disclosed sub-ranges such as

from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 5, from 3 to 5 etc., as well as individual and partial numbers within the recited range, for example, 1, 2, 3, 3.5, 4, and 4.5, unless where integers are required or implicit from context. This applies regardless of the breadth of the disclosed range. Where specific values are required, these will be indicated in the specification.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

Throughout this specification, the term "consisting essentially of", or variations such as "consists essentially of" or "consist essentially thereof", are intended to exclude elements which would materially affect the properties of the claimed composition.

It will be clearly understood that, although a number of prior art publications may be referred to herein, this reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art, in Australia or in any other country or jurisdiction.

Herein the terms "channel" or "channels" may be used interchangeably with "pore" or "pores"

Herein "reference electrode" refers to an electrode having an accurately maintained potential, used as a reference for measurement by other electrodes.

Herein "counter electrode" (which may also be known as an "auxiliary electrode"), refers to an electrode that serves merely to carry the current flowing through an electrochemical cell.

Herein "working electrode" refers to an electrode comprising or consisting of the porous substrate as described herein, where one or more reactions may occur.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. In case of conflict, the present specification, including definitions, will prevail. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

As used herein, the term “treating” (or “treat”, “treatment” etc.), includes a detection, identification reduction, alleviation and/or elimination of one or more symptoms associated with a specific disorder or condition.

As used herein, the term “subject” may be used interchangeably with the terms
5 “patient”, “recipient” and “individual”. In some embodiments, the subject is a mammal. In some embodiments, the subject is a human. In some embodiments the subject is a non-human animal. The subject may be a human, male or female.

Traumatic Brain Injury

In one embodiment, the “traumatic brain injury”, or “TBI” may be a mild,
10 moderate or severe traumatic brain injury. In one embodiment the TBI is a mild TBI, for example a concussion. In one embodiment, the mild TBI may involve one or more symptoms selected from: headache, dizziness, insomnia, decreased concentration and attention span, depression, anxiety, mood swings, impaired balance, decreased speed of information processing, decreased ability to learn new things and recall, and
15 combinations thereof. In one embodiment the TBI is a moderate TBI. The moderate TBI may comprise bleeding. In one embodiment, a moderate TBI may comprise bleeding in the brain and/or a loss of consciousness. In one embodiment the TBI is a severe TBI. A severe TBI may comprise a prolonged unconscious state or coma that lasts days, weeks, or months. Symptoms of severe TBI may include one or more of: loss of consciousness,
20 headache, nausea, vomiting, lack of coordination, dizziness, trouble with balance, dilation of one or more pupils, slurred speech, behavioural or mood changes, loss of coordination, restlessness and agitation, and combinations thereof.

Herein, the TBI may be the result of an “injury to the head” or “head injury”. Herein, “injury to the head” or “head injury” as used interchangeably herein, refers to any
25 trauma to the scalp, skull, or brain. Such injuries may include only a minor bump on the head or may be a serious brain injury. Such injuries may include primary injuries to the brain and/or secondary injuries to the brain. Primary brain injuries occur during the initial insult and result from displacement of the physical structures of the brain. More specifically, a primary brain injury is the physical damage to parenchyma (tissue, vessels)
30 that occurs during the traumatic event, resulting in shearing and compression of the surrounding brain tissue. Secondary brain injuries occur subsequent to the primary injury and may involve an array of cellular processes. More specifically, a secondary brain injury refers to the changes that evolve over a period of time (from hours to days) after the primary brain injury. It includes an entire cascade of cellular, chemical, tissue, or
35 blood vessel changes in the brain that contribute to further destruction of brain tissue.

An injury to the head may be either closed or open (penetrating). A closed head injury refers to a trauma to the scalp, skull or brain where there is no penetration of the skull by a striking object. An open head injury refers a trauma to the scalp, skull or brain where there is penetration of the skull by a striking object. An injury to the head may be caused by physical shaking of a person, by blunt impact by an external mechanical or other force that results in a closed or open head trauma (e.g., vehicle accident such as with an automobile, plane, train, etc.; blow to the head such as with a baseball bat, or from a firearm), a cerebral vascular accident (e.g., stroke), one or more falls (e.g., as in sports or other activities), explosions or blasts (collectively, "blast injuries") and by other types of blunt force trauma. Alternatively, an injury to the head may be caused by the ingestion and/or exposure to a chemical, toxin or a combination of a chemical and toxin. Examples of such chemicals and/or toxins include fires, moulds, asbestos, pesticides and insecticides, organic solvents, paints, glues, gases (such as carbon monoxide, hydrogen sulfide, and cyanide), organic metals (such as methyl mercury, tetraethyl lead and organic tin) and/or one or more drugs of abuse. Alternatively, an injury to the head may be caused as a result of a subject suffering from an autoimmune disease, a metabolic disorder, a brain tumour, hypoxia, a viral infection (e.g., SARS-CoV-2), a fungal infection, a bacterial infection, meningitis, hydrocephalus, or any combinations thereof. In some cases, it is not possible to be certain whether any such event or injury has occurred or taken place. For example, there may be no history on a patient or subject, the subject may be unable to speak, the subject may be aware of what events they were exposed to, etc. Such circumstances may be described as the subject "may have sustained an injury to the head," or as a "suspected injury". In certain embodiments herein, the closed head injury may not include and specifically excludes a cerebral vascular accident, such as stroke.

Herein the TBI may comprise or result in one or more signs and/or symptoms, optionally selected from, but not limited to: headache, convulsions or seizures, blurred or double vision, unequal eye pupil size or dilation, clear fluids draining from the nose or ears, nausea and vomiting, new neurologic deficit, such as slurred speech, weakness of arms, legs, or face; loss of balance, loss of or change in consciousness anywhere from a few seconds to a few hours, decreased level of consciousness (e.g., hard to awaken), mild to profound confusion or disorientation, problems remembering, concentrating, or making decisions, changes in sleep patterns (e.g., sleeping more, difficulty falling or staying asleep); inability to waken from sleep, frustration, irritability, perception/sensation, light-headedness, dizziness, vertigo, or loss of balance or coordination, blurred vision, hearing problems, such as ringing in the ears, bad taste in

the mouth, sensitivity to light or sound, mood changes or swings, agitation, combativeness, or other unusual behaviour, feeling anxious or depressed, fatigue or drowsiness; a lack of energy or motivation, and combinations thereof.

Porous Substrate

- 5 Disclosed herein is a porous substrate for use in detecting nucleic acid analytes, e.g., nucleic acid biomarkers, in complex biological samples. The porous substrate used according to the present disclosure comprises a plurality of channels, wherein:
- at least a portion of the plurality of channels has a mean depth of about 1.0 μm to about 6.0 μm ; and/or
 - 10 - at least a portion of the plurality of channels has a mean diameter or cross section in a range of about 15 nm to about 40 nm,
- wherein optionally, one or more of the following:
- one or more capture probes capable of binding specifically to at least one biomarker, for example at least one nucleic acid analyte, to form
 - 15 a probe-analyte complex; and/or
 - one or more functional groups capable of reacting with an amine group; and/or
 - one or more functional groups capable of forming a covalent bond
 - 20 with a complementary functional groups on one or more capture probes that are capable of binding specifically to at least one biomarker, for example at least one nucleic acid analyte, to form a probe-analyte complex; and/or
 - one or more carboxylic acid groups,
- is disposed on at least one surface of at least a portion of the plurality of channels.
- 25 The porous substrate may comprise one or more “layers”, wherein each layer comprises one or more different chemical functional groups and/or modifications.
- In one embodiment, the porous substrate comprises silicon, wherein the silicon comprises at least one layer which has been modified via at least one of a heat and/or chemical treatment.
- 30 In another embodiment, the porous substrate, optionally comprising, consisting essentially or consisting of silicon, comprises, consists essentially of or consists of a single layer comprising one or more of:

- 5
- one or more capture probes capable of binding specifically to at least one biomarker, for example at least one nucleic acid analyte, to form a probe-analyte complex; and/or
 - one or more functional groups capable of reacting with an amine group; and/or
 - one or more functional groups capable of forming a covalent bond with a complementary functional groups on one or more capture probes that are capable of binding specifically to at least one biomarker, for example at least one nucleic acid analyte, to form a probe-analyte complex; and/or
 - 10 ▪ one or more carboxylic acid groups.

In another embodiment, the porous substrate, optionally comprising, consisting essentially or consisting of silicon, comprises a layer comprising one or more of:

- 15
- one or more capture probes capable of binding specifically to at least one biomarker, for example at least one nucleic acid analyte, to form a probe-analyte complex; and/or
 - one or more functional groups capable of reacting with an amine group; and/or
 - 20 ▪ one or more functional groups capable of forming a covalent bond with a complementary functional groups on one or more capture probes that are capable of binding specifically to at least one biomarker, for example at least one nucleic acid analyte, to form a probe-analyte complex; and/or
 - one or more carboxylic acid groups.

25 In another embodiment, the porous substrate, optionally comprising, consisting essentially or consisting of silicon, comprises at least one layer comprising one or more of:

- 30
- one or more capture probes capable of binding specifically to at least one biomarker, for example at least one nucleic acid analyte, to form a probe-analyte complex; and/or
 - one or more functional groups capable of reacting with an amine group; and/or
 - 35 ▪ one or more functional groups capable of forming a covalent bond with a complementary functional groups on one or more capture probes that are capable of binding specifically to at least one

biomarker, for example at least one nucleic acid analyte, to form a probe-analyte complex; and/or

- one or more carboxylic acid groups.

5 In another embodiment, the porous substrate, optionally comprising, consisting essentially or consisting of silicon, does not comprise or consist essentially of or consist of a plurality of layers comprising one or more of:

- one or more capture probes capable of binding specifically to at least one biomarker, for example at least one nucleic acid analyte, to form a probe-analyte complex; and/or
- 10 ▪ one or more functional groups capable of reacting with an amine group; and/or
- one or more functional groups capable of forming a covalent bond with a complementary functional groups on one or more capture probes that are capable of binding specifically to at least one
- 15 biomarker, for example at least one nucleic acid analyte, to form a probe-analyte complex; and/or
- one or more carboxylic acid groups.

As used herein the terms “probe” or “capture probe” may refer to a capture molecule having sufficient binding properties to specifically bind to a target analyte. For

20 example, a probe may include a polynucleotide having sufficient complementarity to specifically hybridize to a target nucleic acid. In another example, a probe comprises an antibody or a protein tag. A capture probe can function as an affinity-binding molecule for isolation of a target nucleic acid from other nucleic acids and/or components in a mixture. A target nucleic acid can also be specifically bound by a capture probe through

25 intervening molecules such as linkers, adapters and other bridging nucleic acids having sufficient complementarity to specifically hybridize to both a target sequence and a capture probe. The term “capture probe” may also refer to a small organic molecule or polymer having sufficient binding properties to specifically bind to a target nucleic acid analyte.

30 In one embodiment, the capture probe is capable of binding specifically to at least one biomarker, for example at least one nucleic acid analyte, to form a probe-analyte complex, wherein the presence of the probe-analyte complex within a channel causes at least a partial steric and/or charge blockage to at least a portion of the plurality of channels as compared to the plurality of channels in the absence of the probe-analyte

complex. In some embodiments herein, the term “nucleic acid analyte” may be exchanged for “biomarker”.

The choice and number of capture probes can be tailored to target specific analytes. For example, a plurality of the same capture probe may be employed to increase selectivity for a single nucleic acid analyte. In other examples, a plurality of the different capture probes may be employed, each capable of binding specifically to a different nucleic acid analyte, to enable detection of multiple nucleic acid analytes simultaneously. In the case of the latter, this may be advantageous where a plurality of biomarkers are used to diagnose a single injury or medical condition (e.g., a panel of biomarkers). Alternatively, a plurality of different capture probes may be used to detect a plurality of nucleic acid analytes, each associated with a different injury or medical condition, thereby permitting screening for multiple injuries or conditions simultaneously. In some of the examples disclosed herein, the porous substrate and/or electrodes as described herein can be produced or designed to be replaceable in an article or device as described herein.

The plurality of channels may be of various shapes, including circular, substantially circular, oval, substantially oval, regular and/or irregular in shape. The shape of the plurality of channels may be substantially the same across the porous substrate or the shape may vary at different portions of the porous substrate. For at least a portion of the plurality of channels, the shape of the cross section may be consistent, substantially consistent, or vary through one or more channels, for example along a particular dimension, such the depth.

In one embodiment at least a portion of the plurality of channels has a mean depth of about 1.0 μm to about 6.0 μm . For example a mean depth in a range of about 1.0 μm to about 2.0 μm , or about 1.0 μm to about 4.0 μm . In another embodiment at least a portion of the plurality channels has a mean diameter or cross section in a range of about 15 nm to about 40 nm. In yet another embodiment, at least a portion of the plurality of channels has a mean depth of about 1.0 μm to about 6.0 μm , and at least a portion of the plurality of channels has a mean diameter or cross section in a range of about 15 nm to about 40 nm. For example, at least a portion of the plurality of channels has a mean depth of about 1.0 μm to about 6.0 μm , and a mean diameter or cross section in a range of about 15 nm to about 40 nm.

In one embodiment, the mean depth of at least a portion of the plurality of channels is about, or at least about: 1.00 μm , 1.10 μm , 1.20 μm , 1.30 μm , 1.40 μm , 1.50 μm , 1.60 μm , 1.70 μm , 1.80 μm , 1.90 μm , 2.00 μm , 2.10 μm , 2.20 μm , 2.30 μm , 2.40 μm , 2.50

μm, 2.60 μm, 2.70 μm, 2.80 μm, 2.90 μm, 3.00 μm, 3.10 μm, 3.20 μm, 3.30 μm, 3.40 μm, 3.50 μm, 3.60 μm, 3.70 μm, 3.80 μm, 3.90 μm, 4.00 μm, 4.10 μm, 4.20 μm, 4.30 μm, 4.40 μm, 4.50 μm, 4.60 μm, 4.70 μm, 4.80 μm, 4.90 μm, 5.00 μm, 5.10 μm, 5.20 μm, 5.30 μm, 5.40 μm, 5.50 μm, 5.60 μm, 5.70 μm, 5.80 μm, 5.90 μm, or 6.00 μm. In
5 another embodiment, the mean depth of at least a portion of the plurality of channels is less than about: 6.00 μm, 5.90 μm, 5.80 μm, 5.70 μm, 5.60 μm, 5.50 μm, 5.40 μm, 5.30 μm, 5.20 μm, 5.10 μm, 5.00 μm, 4.90 μm, 4.80 μm, 4.70 μm, 4.60 μm, 4.50 μm, 4.40 μm, 4.30 μm, 4.20 μm, 4.10 μm, 4.00 μm, 3.90 μm, 3.80 μm, 3.70 μm, 3.60 μm, 3.50 μm, 3.40 μm, 3.30 μm, 3.20 μm, 3.10 μm, 3.00 μm, 2.90 μm, 2.80 μm, 2.70 μm, 2.60
10 μm, 2.50 μm, 2.40 μm, 2.30 μm, 2.20 μm, 2.10 μm, 2.00 μm, 1.90 μm, 1.80 μm, 1.70 μm, 1.60 μm, 1.50 μm, 1.40 μm, 1.30 μm, 1.20 μm, or 1.10 μm. In yet another embodiment, the mean depth of at least a portion of the plurality of channels may be in a range of any of the aforementioned values.

In one embodiment, at least about 10% of the plurality of channels have a mean
15 depth in a range of about 1.0 μm to about 6.0 μm. For example, about, or at least about: 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95 %, of the plurality of channels have a mean depth in a range of about 1.0 μm to about 6.0 μm. This may be measured by any appropriate technique known in the art, for example scanning electron microscopy (SEM).

20 Alternatively or in addition to at least a portion of the plurality of channels having a mean depth in a range of about 1.0 μm to about 6.0 μm as described hereinabove, at least a portion of the plurality of channels may have a mean diameter or cross section in a range of about 15 nm to about 40 nm. For example in a range of about 15 nm to about 30 nm, or about 20 nm to about 30 nm. In one embodiment, the mean diameter or cross
25 section of at least a portion of the plurality of channels is about, or at least about: 15.00 nm, 15.50 nm, 16.00 nm, 16.50 nm, 17.00 nm, 17.50 nm, 18.00 nm, 18.50 nm, 19.00 nm, 19.50 nm, 20.00 nm, 20.50 nm, 21.00 nm, 21.50 nm, 22.00 nm, 22.50 nm, 23.00 nm, 23.50 nm, 24.00 nm, 24.50 nm, 25.00 nm, 25.50 nm, 26.00 nm, 26.50 nm, 27.00 nm, 27.50 nm, 28.00 nm, 28.50 nm, 29.00 nm, 29.50 nm, 30.00 nm, 30.50 nm, 31.00 nm,
30 31.50 nm, 32.00 nm, 32.50 nm, 33.00 nm, 33.50 nm, 34.00 nm, 34.50 nm, 35.00 nm, 35.50 nm, 36.00 nm, 36.50 nm, 37.00 nm, 37.50 nm, 38.00 nm, 38.50 nm, 39.00 nm, or 39.50 nm. In another embodiment, the mean diameter or cross section of at least a portion of the plurality of channels is less than about: 40.00 nm, 39.50 nm, 39.00 nm, 38.50 nm, 38.00 nm, 37.50 nm, 37.00 nm, 36.50 nm, 36.00 nm, 35.50 nm, 35.00 nm, 34.50 nm,
35 34.00 nm, 33.50 nm, 33.00 nm, 32.50 nm, 32.00 nm, 31.50 nm, 31.00 nm, 30.50 nm,

30.00 nm, 29.50 nm, 29.00 nm, 28.50 nm, 28.00 nm, 27.50 nm, 27.00 nm, 26.50 nm, 26.00 nm, 25.50 nm, 25.00 nm, 24.50 nm, 24.00 nm, 23.50 nm, 23.00 nm, 22.50 nm, 22.00 nm, 21.50 nm, 21.00 nm, 20.50 nm, 20.00 nm, 19.50 nm, 19.00 nm, 18.50 nm, 18.00 nm, 17.50 nm, 17.00 nm, 16.50 nm, 16.00 nm, or 15.50 nm. In yet another
5 embodiment, the mean diameter or cross section of at least a portion of the plurality of channels may be in a range of any of the aforementioned values.

In one embodiment, at least about 10% of the plurality of channels have a mean diameter or cross section in a range of about 15 nm to about 40 nm. For example, about, or at least about: 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%,
10 70%, 75%, 80%, 85%, 90%, or 95 %, of the plurality of channels have a mean diameter or cross section in a range of about 15 nm to about 40 nm. This may be measured by any appropriate technique known in the art, for example SEM.

The overall dimensions of the porous substrate used according to the present disclosure may vary, and can be dictated by the final application or use of the porous
15 substrate, for example based on any samples which are used in conjunction with the porous substrate and/or in a device where the porous substrate is present as a component, for example as part of an electrode, electrochemical sensor and/or device.

The porous substrate may comprise, consist essentially of, or consist of any appropriate conducting or semi-conducting material, in any form. Examples of suitable
20 forms includes, but is not limited to: wires, rods, discs, foils, wafers or chips. The conducting or semi-conducting material may be in the form of a coating deposited on another substrate, such as a non-conducting substrate, by any known deposition process. Suitable materials include, but are not limited to: platinum, gold, silver, cobalt, gallium, indium, tin, vanadium, zirconium, copper, aluminium, iridium, palladium, rhodium,
25 silicon, zinc, iron, steel, brass, carbon (for example graphite), alloys, oxides, or metallic compounds of these elements, and the like, and mixtures thereof. Examples of semi-conductors include, but are not limited to: a silicon semi-conductor, a germanium semi-conductor and an indium tin oxide semi-conductor, and mixtures thereof, for example a silicon-germanium mixed semi-conductor. The semi-conductor may be an n-type or p-
30 type doped semi-conductor (for example a silicon semi-conductor). If dopants are present, they may be a conventional n-type dopant such as phosphorous, boron or antimony.

In one embodiment the porous substrate comprises a wafer. In another embodiment the porous substrate comprises silicon, optionally a silicon wafer. In yet another

embodiment, at least a portion of the silicon, for example silicon wafer, may be modified via chemical and or physical treatment.

In one embodiment, the porous substrate is not in the form of a film.

In one embodiment, the porous substrate does not comprise, or does not consist of
5 nanoparticles.

In one embodiment, the porous substrate does not comprise one or more of: aluminium, platinum, gold and/or silver. In another embodiment the porous substrate does not comprise an alloy comprising gold and /or silver.

In one embodiment the porous substrate comprises, consists essentially of, or
10 consists of, a material selected from silicon. The silicon may be doped, and be a n or p type silicon.

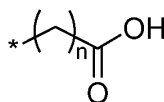
The surface of the porous substrate can be modified to introduce and/or immobilise compounds (optionally one or more capture probes), and/or functional groups. For example, linkers, functional groups and/or capture probes can be joined to the porous
15 substrate, directly or indirectly, by any means known in the art, including covalent and non-covalent interactions, or any combination thereof (see, e.g., Chan et al., 2007, PLoS One 2:e1164; Cazalis et al., Bioconj. Chem. 15:1005-1009; Soellner et al., 2003, J. Am. Chem. Soc. 125:11790-11791; Sun et al., 2006, Bioconjug. Chem. 17:52-57; Decreau et al., 2007, J. Org. Chem. 72:2794-2802; Camarero et al., 2004, J. Am. Chem. Soc.
20 126:14730-14731; Girish et al., 2005, Bioorg. Med. Chem. Lett. 15:2447-2451; Kalia et al., 2007, Bioconjug. Chem. 18:1064-1069; Watzke et al., 2006, Angew. Chem. Int. Ed. Engl. 45:1408-1412; Parthasarathy et al., 2007, Bioconjugate Chem. 18:469-476; and Bioconjugate Techniques, G. T. Hermanson, Academic Press (2013), and are each hereby incorporated by reference in their entirety). Any suitable molecule or materials
25 may be employed for this purpose, including proteins, nucleic acids, carbohydrates and small molecules. For example, in one embodiment a capture probe or a surface of the substrate comprises an azide group which can react with an alkynyl group on the other entity to facilitate association or binding.

The surface of the porous substrate can be modified using methods referred to as
30 "click chemistry". Exemplary reactions include the copper catalyzed reaction of an azide and alkyne to form a triazole (Huisgen 1, 3-dipolar cycloaddition), strain-promoted azide alkyne cycloaddition (SPAAC), reaction of a diene and dienophile (Diels-Alder), strain-promoted alkyne-nitrone cycloaddition, reaction of a strained alkene with an azide, tetrazine or tetrazole, alkene and azide [3+2] cycloaddition, alkene and tetrazine inverse

electron demand Diels-Alder (IEDDA) reaction (e.g., m-tetrazine (mTet) or phenyl tetrazine (pTet) and trans-cyclooctene (TCO); or pTet and an alkene), alkene and tetrazole photoreaction, Staudinger ligation of azides and phosphines, and various displacement reactions, such as displacement of a leaving group by nucleophilic attack
 5 on an electrophilic atom (for example, Horisawa, *Front Physiol* (2014). 5: 457; Knall, Hollauf et al., *Tetrahedron Lett* (2014) 55(34): 4763-4766). Exemplary displacement reactions include reaction of an amine with: an activated ester; an N-hydroxysuccinimide ester; an isocyanate; an isothiocyanate, an aldehyde, an epoxide, or the like.

The porous substrate used according to the present disclosure may comprise one or
 10 more functional groups that are able to react with one or more complementary functional groups on a capture probe, and/or a linking compound (or “tether”), which can be subsequently modified or used to immobilise at least one capture probe to the substrate. Examples of functional groups may independently selected from, but not limited to: α,β -unsaturated carbonyl, acid, acyl halide, acylazide, haloimide, alcohol, aldehyde, alkene,
 15 alkyne, amide, amine, azide, aziridine, biotin or thiirane functional group with a complementary reactive group, carbocyclic acid, disulfide, epoxide, ester, activated ester (e.g., N-hydroxysuccinimide ester, pentynoic acid STP ester), halide, heteroaryl group, heterocyclic or heteroaryl group, hydrazide, hydrazine, hydrazone, imidoester, imine, isocyanate, isothiocyanate, ketone, maleimide, nitrile, nitron, oxime, phosphine,
 20 sulfhydryl, sulfinic acid, sulfonic acid, sulfonyl halide, tetrazine, tetrazole, thioester, triazolyl, or mixtures thereof.

For example, in one embodiment, one or more carboxylic acid groups are disposed on at least one surface of at least a portion of the plurality of channels. The carboxylic acid groups may be derived from the attachment of one or more compounds comprising
 25 at least one carboxylic acid groups and at least one other functional group which can react with a complimentary functional group on at least one surface of at least a portion of the plurality of channels. In another embodiment, one or more carboxylic acid groups of Formula (I) is disposed on at least one surface of at least a portion of the plurality of channels:



30

Formula I

wherein n is an integer of 1 to 15, and * denotes attachment to the at least one surface on a portion of the plurality of channels. For example, n may be: 1, 2, 3, 4, 5, 6,

7, 8, 9, 10, 11, 12, 13, 14 or 15. In one embodiment a plurality of carboxylic acid groups of Formula (I) may be disposed on at least one surface of at least a portion of the plurality of channels, where n is the same or different.

5 In one embodiment, one or more functional groups capable of reacting with an amine group are disposed on at least one surface of at least a portion of the plurality of channels. The reaction may comprise the formation of one or more covalent bonds. Examples of functional groups capable of reacting with an amine group include, but are not limited to: an aldehyde group, an ester (optionally succinimidyl ester), an acid anhydride, an alkyl halide, an acid halide, a ketone, an epoxy, a carboxylic acid,
10 isocyanates, isothiocyanates, fluorophenyl ester, and mixtures thereof.

In one embodiment at least a portion of the porous substrate, for example a layer, is modified. The modification may be made to protect underlying sections/portions of the porous substrate from degradation, for example to minimise or reduce degradation when the porous substrate, or part thereof, is exposed to an aqueous medium; and/ or to
15 increase conductivity of the porous substrate, or portion thereof. The porous substrate may be exposed to heat and/or a source of carbon (e.g., acetylene), optionally in the presence of a gas (for example nitrogen), for a period of time. In one embodiment the porous substrate is modified such that it comprises 1, 2, 3, 4 or 5 physically and/or chemically distinct layers.

20 In one embodiment, one or more capture probes are disposed on at least one surface of at least a portion of the plurality of channels. In another embodiment, the one or more capture probes are capable of binding at least one nucleic acid analyte. A skilled person will appreciate that the choice of capture probe will depend on the type of nucleic acid analyte to be detected. Examples of nucleic acid analytes include, but are not limited to:
25 a DNA analyte, a messenger RNA (mRNA) analyte, ribosomal RNA (rRNA) analyte, a transfer RNA (tRNA) analyte, a microRNA (miRNA) analyte, a short interfering RNA (siRNA) analyte, plasmid DNA (pDNA) and mixtures thereof. In another embodiment, at least one capture probe is a DNA capture probe or a peptide nucleic acid probe (PNA) probe.

30 In accordance with an example in which the capture probe is a DNA capture probe, the probe may comprise a polynucleotide sequence of between about 15 and about 70 nucleotides in length which is sufficiently complementary to a sequence of corresponding length in a nucleic acid analyte to be detected (e.g., DNA or RNA), such that the capture probe hybridises to a sequence in the nucleic acid analyte. For example,
35 the DNA capture probe may be: 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29,

30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69 or 70 nucleotides in length. As used herein, the term “sufficiently complementary” as used in the context of capture probe and nucleic acid analyte is intended to indicate a sufficient degree of complementarity or precise pairing such that stable and specific binding occurs between nucleic acid sequences e.g., between a capture probe and to a sequence within the nucleic acid analyte (e.g., DNA or RNA). It is understood that the sequence of a probe need not be 100% complementary to that of its target nucleic acid analyte. The term encompasses a sequence complementary to another sequence with the exception of mismatches which do not prevent hybridisation and subsequent formation of a duplex. In some cases, the probe is complementary to a sequence within the target nucleic acid analyte with the exception of 1-2 mismatches. In some cases, the sequences are complementary except for 1 mismatch. In some cases, the sequences are complementary except for 2 mismatches. In other cases, the sequences are complementary except for 3 mismatches. In yet other cases, the sequences are complementary except for 4 mismatches. Preferably, there is sufficient complementarity between the capture probe and the nucleic acid analyte such that hybridisation can occur at a temperature that does not significantly vary to the temperature of the porous substrate.

As used in the context of nucleic acid binding, the term “hybridise”, “hybridising” or similar refers to a process whereby two complementary or substantially complementary nucleic acid sequences anneal to each in accordance with Watson-Crick base pairing rules.

Herein, partial steric and/or charge blockage of at least a portion of the plurality of channels by a probe-analyte complex can be capable of reducing diffusion of a redox species through the channel. The redox species may be a transition metal species, wherein the transition metal can adopt two valence states (e.g., a metal ion (M) being able to adopt M(II) and M(III) states). In some embodiments, the redox probe contains a metal ion, wherein the metal of the metal ion may be selected from, but not limited to: iron, ruthenium, iridium, osmium, cobalt, tungsten and molybdenum. Examples of redox species include, but are not limited to: $\text{Fe}(\text{CN})_6^{3-/4-}$, $\text{Fe}(\text{NH}_3)_6^{3+/2+}$, $\text{Fe}(\text{phen})_3^{3+/2+}$, $\text{Fe}(\text{bipy})_2^{3+/2+}$, $\text{Fe}(\text{bipy})_3^{3+/2+}$, $\text{Ru}_3^{3+/2+}$, $\text{RuO}_4^{3-/2-}$, $\text{Ru}(\text{CN})_6^{3-/4-}$, $\text{Ru}(\text{NH}_3)_6^{3+/2+}$, $\text{Ru}(\text{en})_3^{3+/2+}$, $\text{Ru}(\text{NH}_3)_5(\text{Py})^{3+/2+}$, $\text{Ir}^{4+/3+}$, $\text{Ir}(\text{Cl})_6^{2-/3-}$, $\text{Ir}(\text{Br})_6^{2-/3-}$, $\text{Os}(\text{bipy})_2^{3+/2+}$, $\text{Os}(\text{bipy})_3^{3+/2+}$, $\text{OsCl}_6^{2-/3-}$, $\text{Co}(\text{NH}_3)_6^{3+/2+}$, $\text{W}(\text{CN})_6^{3-/4-}$, $\text{Mo}(\text{CN})_6^{3-/4-}$, optionally substituted ferrocene, polyferrocene, quinones, such as p-benzoquinone and hydroquinone and phenol. In specific embodiments, the redox species is an iron-

containing species in which iron is in Fe(II) and/or Fe(III) states. The redox species may be present in the biological sample in an amount of from 0.1 mM to 100 mM, optionally from 0.5 mM to 10 mM, optionally from 0.5 mM to 2 mM, optionally from 0.5 mM to 1.5 mM, optionally about 1 mM.

5 In one embodiment, the porous substrate is or may be used to detect at least one nucleic acid analyte in a complex biological sample, optionally wherein the nucleic acid analyte is selected from the group comprising, but not limited to: a messenger RNA (mRNA) analyte, ribosomal RNA (rRNA), a transfer RNA (tRNA), a microRNA (miRNA), a short interfering RNA (siRNA), and mixtures thereof. The at least one
10 nucleic acid analyte may be associated with a disease state or injury. One exemplary injury with which the porous substrate may be used is a brain injury, optionally a traumatic brain injury (TBI). Exemplary nucleic acid analytes for use in detecting TBI include the microRNAs described in PCT/US2015/051518, the full contents of which is incorporated herein in its entirety. For example, the miRNA associated with TBI may be
15 selected from miR-142-3p, miR-196b-5p, let-7f-5p, miR-150-5p, miR-196b-5p and combination thereof. Exemplary capture probes for detecting the aforementioned miRNA are described in Table 1 in the Examples herein. However, a skilled person will appreciate that the porous substrate may be used to detect nucleic acid analytes associate with any injury, condition or disease, including but not limited to: cancers, kidney disease
20 (including acute kidney injury), cardiovascular disease, autoimmune diseases, infectious diseases, metabolic disease, liver disease and diseases of the blood. Furthermore, the porous substrate may be used to detect nucleic acid analytes associated with pathogens and/or contaminants of interest. Suitable pathogens include but are not limited to, viruses, bacteria, algae, fungi, prions or protozoa. The pathogen may cause a disease or condition
25 to a human or non-human animal. In other examples, the pathogen may be a plant pathogen.

 In one embodiment, the porous substrate may be used to detect at least one nucleic acid analyte in a liquid sample. The liquid sample may comprise or consist of biological sample or media, for example a complex biological sample or media. In one example,
30 the liquid sample is a bodily fluid. Bodily fluids of interest include, but are not limited to one or more of: whole blood, fractioned blood, serum, plasma, urine, saliva, sweat, spinal fluid, mucus (including nasal drainage and phlegm), amniotic fluid, aqueous humor, vitreous humor, breast milk, cerebrospinal fluid (CSF), pericardial fluid, peritoneal fluid, pleural fluid, pus, rheum, saliva, sebum (skin oil), semen, sputum, sweat, synovial fluid,
35 tears, vomit, urine and exhaled condensate. In other examples, the liquid sample is a cell

lysate, cell culture medium, and mixtures thereof. In certain examples, porous substrate may be used to detect at least one nucleic acid analyte in a liquid sample directly. In other examples, the liquid sample may be subjected to one or more processes (e.g., dilution, lysis, fractionation), prior to detection with the porous substrate or device comprising same. In some of the examples disclosed herein describing biological liquid samples, the sample may be obtained from a mammalian subject, such as a human.

In accordance with other examples in which the porous substrate of the disclosure is used to detect a nucleic acid analyte associated with an allergen or contaminant, the sample may be a biological or organic sample selected from, but not limited to: a soil sample, a water sample, a plant material, a food or beverage sample, an agricultural product, surface debris, a surface swab or any combination thereof. In some of the examples disclosed herein, the biological or organic sample may be obtained in a liquid form or processed so to a liquid form for use with the porous substrate.

The liquid samples described herein may be used and/or tested directly, for example using the substrate, electrode, electrochemical cell and/or device as described herein, or may be diluted prior to being used and/or tested. For example the samples may be diluted to be about, at least about, or less than about: 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98% of an original concentration (for example w/w, w/v or v/v). In accordance with an example in which the sample is diluted to be about 2% concentration, the diluted sample may comprise about 2% of the original sample and 98% of a diluent, such as a buffer (for example w/w, w/v or v/v). Likewise, an example in which the sample is diluted to be about 5% concentration, the diluted sample may comprise about 2% of the original sample and 95% of a diluent, such as a buffer (for example w/w, w/v or v/v). Other dilutions may be determined based on this approach.

Herein, the liquid sample may also refer to a liquid comprising or consisting essentially of water (optionally sea water, waste water or fresh water), for example for water testing. Alternatively, the liquid sample may also be an environmental sample, for example as part of bioremediation testing. The sample may comprise or consist of effluent, which could form part of any water testing. Alternatively the liquid sample comprise or consist essentially of plant and/or animal products, which may find application in areas such as biosecurity.

In one embodiment, the porous substrate used according to the present disclosure is capable of detecting at least one nucleic acid analyte in a sample. In another embodiment, the porous substrate is capable of detecting at least one nucleic acid analyte

associated with a disease state or injury, such as a brain injury, optionally a TBI, in a sample. In another embodiment, the porous substrate is capable of detecting a plurality of nucleic acid analytes, wherein the plurality of nucleic acid analytes are optionally associated with a disease state or injury, such as a brain injury, optionally a TBI, in a sample. In yet another embodiment, the porous substrate is capable of detecting at least one nucleic acid analyte in a sample at a concentration of about 1 pM or less.

In one embodiment the porous substrate is capable of detecting and/or quantifying at least one nucleic acid analyte in a sample within: about 60 minutes, 30 minutes or 20 minutes of contacting the sample. For example the detection of the at least one nucleic acid analyte may be possible within about: 1 minute, 2 minutes, 3 minutes, 4 minutes, 5 minutes, 6 minutes, 7 minutes, 8 minutes, 9 minutes, 10 minutes, 11 minutes, 12 minutes, 13 minutes, 14 minutes, 15 minutes, 16 minutes, 17 minutes, 18 minutes, 19 minutes, 20 minutes, 21 minutes, 22 minutes, 23 minutes, 24 minutes, 25 minutes, 26 minutes, 27 minutes, 28 minutes, 29 minutes, 30 minutes, 35 minutes, 40 minutes, 45 minutes, 50 minutes, 55 minutes, or about 60 minutes.

Electrode

Disclosed herein is an electrode comprising the porous substrate as defined herein for use in detecting a nucleic acid analyte in complex biological samples.

In one embodiment, the electrode comprises:

- a working electrode comprising the porous substrate as defined herein;
- a reference electrode; and
- a counter electrode.

In one embodiment the reference electrode comprises, consists essentially of, or consists of, at least one material selected from, but not limited to: silver, silver salts (optionally silver halides), calomel, and mixtures thereof. In another embodiment the reference electrode may be a hydrogen electrode.

In one embodiment, the counter electrode comprises, consists essentially of, or consists of, at least one material selected from, but not limited to: carbon, graphite, platinum, and mixtures thereof.

The electrode may be integrated into an article or device utilising appropriate techniques in the art, for example screen printing, spray coating, inkjet printing, and mixtures thereof.

The electrode can be tailored to target specific analytes, for example nucleic acid analytes. The electrode may be in the form of a disposable article, wherein it can be integrated/used in a device as described herein, and then potentially replaced with the same type of electrode (e.g., to test a new sample), or with a different electrode comprising different capture probes (e.g., to test the sample or different sample for the presence of one or more different nucleic acid analytes).

In another embodiment, an insulating material is disposed on at least a portion of the electrode. For example the insulating materials may be disposed on about, at least about, or less than about: 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95 % of the electrode. Examples of insulating materials include, but are not limited to: silicon dioxide; a polymer, a glass, a ceramic, or the like. For example, the insulating material may be polyvinyl chloride, polycarbonate, an epoxy, polyester, paper, cardboard, ceramic, ceramic-coated metal, polydimethylsiloxanes and blends of these materials (e.g., a blend of polycarbonate and polyester).

In one embodiment the electrode is used in conjunction with a device, for example electrochemical analysis devices that are able to scan a potential within a certain range and measure current, such as potentiostats.

In one embodiment the electrode further comprises a coating. The coating may be a polymer, for example polydimethylsiloxane or an epoxy based polymer, and mixtures thereof. The coating may be applied by any method known in the art.

In one embodiment, the electrode comprises an area suitable for depositing a liquid, for example a complex biological samples or liquid sample as described herein, wherein the area suitable for depositing a liquid is optionally in the form of a reservoir, for example a reservoir formed in a coating or in the porous substrate per se. In yet another embodiment, the area for depositing the liquid is in contact with one or more of the: working electrode, reference electrode; and/or counter electrode.

In one embodiment, the electrode is capable of detecting at least one nucleic acid analyte in a sample. The at least one nucleic acid analyte may be associated with a condition, disease state or injury, such as a brain injury, optionally a TBI. However, other conditions, diseases or injuries are contemplated and described herein in the context of the porous substrate. In another embodiment, the electrode is capable of detecting a plurality of nucleic acid analytes, wherein the plurality of nucleic acid analytes are optionally associated with a disease state or injury, such as a brain injury, optionally a

TBI, in a sample. In yet another embodiment, the electrode is capable of detecting at least one nucleic acid analyte in a sample at a concentration of about 1 pM or less.

In one embodiment the electrode is capable of detecting at least one nucleic acid analyte in a sample within about: 60 minutes, 30 minutes or 20 minutes of contacting the sample. For example the detection of the at least one analyte may be possible within about: 1 minute, 2 minutes, 3 minutes, 4 minutes, 5 minutes, 6 minutes, 7 minutes, 8 minutes, 9 minutes, 10 minutes, 11 minutes, 12 minutes, 13 minutes, 14 minutes, 15 minutes, 16 minutes, 17 minutes, 18 minutes, 19 minutes, 20 minutes, 21 minutes, 22 minutes, 23 minutes, 24 minutes, 25 minutes, 26 minutes, 27 minutes, 28 minutes, 29 minutes, 30 minutes, 35 minutes, 40 minutes, 45 minutes, 50 minutes, 55 minutes, or about 60 minutes.

In one embodiment the electrode is used to detect at least one nucleic acid analyte in a sample, wherein the nucleic acid analyte is optionally associated with a disease state or injury, such as a brain injury, optionally a TBI. In another embodiment, the nucleic acid analyte is selected from, but not limited to: a messenger RNA (mRNA), ribosomal RNA (rRNA), a transfer RNA (tRNA), a microRNA (miRNA), a short interfering RNA (siRNA), and mixtures thereof.

Electrochemical cell

Disclosed herein is an electrochemical cell comprising the electrode as defined herein for use in detecting a nucleic acid analyte in complex biological samples.

The electrochemical cell may be an electrochemical sensor. In one embodiment, the electrochemical cell is capable of detecting at least one nucleic acid analyte in a sample. In another embodiment, the electrochemical cell is capable of detecting at least one nucleic acid analyte associated with a disease state or injury, such as a brain injury, optionally a TBI, in a sample. However, other conditions, diseases or injuries are contemplated and described herein in the context of the porous substrate. In another embodiment, the electrochemical cell is capable of detecting a plurality of nucleic acid analytes, wherein the plurality of nucleic acid analytes are optionally associated with a disease state or injury, such as a brain injury, optionally a TBI, in a sample. In yet another embodiment, the electrochemical cell is capable of detecting at least one nucleic acid analyte in a sample at a concentration of at least about 1 pM.

In one embodiment the electrochemical cell is capable of detecting at least one nucleic acid analyte in a sample within about: 60 minutes, 30 minutes or 20 minutes of contacting the sample. For example the detection of the at least one analyte may be

possible within about: 1 minute, 2 minutes, 3 minutes, 4 minutes, 5 minutes, 6 minutes, 7 minutes, 8 minutes, 9 minutes, 10 minutes, 11 minutes, 12 minutes, 13 minutes, 14 minutes, 15 minutes, 16 minutes, 17 minutes, 18 minutes, 19 minutes, 20 minutes, 21 minutes, 22 minutes, 23 minutes, 24 minutes, 25 minutes, 26 minutes, 27 minutes, 28 minutes, 29 minutes, 30 minutes, 35 minutes, 40 minutes, 45 minutes, 50 minutes, 55 minutes, or about 60 minutes.

In one embodiment, the electrochemical cell is used to detect at least one nucleic acid analyte in a sample, wherein the nucleic acid analyte is optionally associated with a disease state or injury, such as a brain injury, optionally a TBI. In another embodiment, the nucleic acid analyte is selected from, but not limited to: a messenger RNA (mRNA), ribosomal RNA (rRNA), a transfer RNA (tRNA), a microRNA (miRNA) and a short interfering RNA (siRNA).

Device

Disclosed herein is a device for use in detecting a nucleic acid analyte in complex biological samples, the device comprising the porous substrate as described herein, the electrode as described herein and/or the electrochemical cell as described herein .

In one embodiment, the device is an electrochemical biosensor capable of detecting at least one nucleic acid analyte in a sample. For example the device may be a diagnostic device, for example a point of care device. Herein, the phrase “electrochemical biosensor” may be exchanged with “electrochemical sensor” and *vice versa*.

In one embodiment, the device is capable of detecting at least one nucleic acid analyte in a sample. In another embodiment, the device is capable of detecting at least one nucleic acid analyte associated with a disease state or injury, such as a brain injury, optionally a TBI, in a sample. However, other conditions, diseases or injuries are contemplated and described herein in the context of the porous substrate. In another embodiment, the device is capable of detecting a plurality of nucleic acid analytes, wherein the plurality of nucleic acid analytes are optionally associated with a disease state or injury, such as a brain injury, optionally a TBI, in a sample. In yet another embodiment, the device is capable of detecting at least one nucleic acid analyte in a sample at a concentration of at least about 1 pM.

In one embodiment the device is capable of detecting at least one nucleic acid analyte in a sample within about: 60 minutes, 30 minutes or 20 minutes of contacting the sample. For example the detection of the at least one analyte may be possible within about: 1 minute, 2 minutes, 3 minutes, 4 minutes, 5 minutes, 6 minutes, 7 minutes, 8

minutes, 9 minutes, 10 minutes, 11 minutes, 12 minutes, 13 minutes, 14 minutes, 15 minutes, 16 minutes, 17 minutes, 18 minutes, 19 minutes, 20 minutes, 21 minutes, 22 minutes, 23 minutes, 24 minutes, 25 minutes, 26 minutes, 27 minutes, 28 minutes, 29 minutes, 30 minutes, 35 minutes, 40 minutes, 45 minutes, 50 minutes, 55 minutes, or
5 about 60 minutes.

In one embodiment, the device is used to detect at least one nucleic acid analyte in a sample, wherein the nucleic acid analyte is optionally associated with a disease state or injury, such as a brain injury, optionally a TBI. In another embodiment, the nucleic acid analyte is selected from, but not limited to: a messenger RNA (mRNA), ribosomal RNA
10 (rRNA), a transfer RNA (tRNA), a microRNA (miRNA) and a short interfering RNA (siRNA).

The device may be in a form that is suitable for deployment within or outside of a clinical setting, for example within an urban hospital or a field hospital, or within a rural environment, and/or in other settings, for example a clinical setting. The device may
15 provide one or more of: assistance in clinical and/or operational decision making, for example in relation to the diagnosis and/or treatment of a subject; to contribute to achieving improved short term and long term outcomes based on the results obtained from a device; to achieve an analysis in a short period of time (for example about or less than about 60, 40, 30 or 20 minutes); determining the concentration of one or more
20 analytes, in accessible physiological media (for example a complex biological sample or a liquid sample as described herein); ease of operation, without the need to undergo long-term or extensive training requirements.

Disclosed herein is use of the porous substrate as described herein, the electrode as described herein, the electrochemical cell as described herein, and/or the device as
25 described herein, to detect a nucleic acid analyte in a sample. In some examples, the nucleic acid analyte is associated with a disease state or injury, such as a brain injury, optionally a TBI.

Applications and uses

The porous substrate, electrode, electrochemical cell and/or device may be used to
30 detect a nucleic acid analyte or biomarker in a complex biological sample or media. Accordingly, the present disclosure provides a method of detecting at least one biomarker, for example at least one nucleic acid analyte, in a complex biological sample, the method comprising:

- (i) introducing the complex biological sample to an electrochemical biosensor comprising a porous substrate comprising a plurality of channels, wherein:
- at least a portion of the plurality of channels has a mean depth of about 1.0 μm to about 6.0 μm ; and/or
 - 5 - at least a portion of the plurality of channels has a mean diameter or cross section in a range of about 15 nm to about 40 nm,
- wherein one or more capture probes capable of binding specifically to at least biomarker, for example at least one nucleic acid analyte, to form a probe-analyte complex are disposed on at least one surface of at least a portion of at least a portion of the plurality
- 10 of channels; and
- (ii) determining the presence or absence of the at least one biomarker, for example the at least one nucleic acid analyte, in the sample, optionally based on measurements of peak current obtained from differential pulse voltammograms prior to and after introducing the complex biological sample to the
- 15 electrochemical biosensor, wherein detection of a change in the peak current after introduction of the complex biological sample to the electrochemical biosensor indicates the presence of the at least one biomarker, for example the at least one nucleic acid analyte, in the complex biological sample and detection of no change in the peak current after introduction of the complex biological sample to the
- 20 electrochemical biosensor indicates that the at least one biomarker, for example the at least one nucleic acid analyte, is not present in the complex biological sample above a threshold level.

As used herein, the terms “complex biological sample”, “complex biological media” or similar are intended to mean heterogeneous biological samples or media

25 containing nucleic acids, proteins and/or cellular materials of varying molecular weights and/or origins. In some examples, a complex biological sample or media which may be subjected to a method of detection of the disclosure is a clinically-relevant biological sample. Exemplary complex biological samples of clinical relevance are described herein, and include, without limitation, whole blood, fractioned blood, serum, plasma,

30 urine, saliva, sweat, spinal fluid, mucus (including nasal drainage and phlegm), amniotic fluid, aqueous humor, vitreous humor, breast milk, cerebrospinal fluid (CSF), pericardial fluid, peritoneal fluid, pleural fluid, pus, rheum, saliva, sebum (skin oil), semen, sputum, sweat, synovial fluid, tears, vomit, urine, exhaled condensate, cell lysate, cell culture medium, and mixtures thereof. In other examples, a complex biological sample or media

35 which may be subjected to a method of detection of the disclosure is an environmentally-

relevant biological sample. Exemplary complex biological samples of environmental relevance are described herein, and include, without limitation, water samples, effluent, soil samples and mixtures thereof. In yet other examples, a complex biological sample or media which may be subjected to a method of detection of the disclosure is an agriculturally-relevant biological sample. Exemplary complex biological samples of agricultural relevance are described herein, and include, without limitation, plant and/or animal products or tissues, including products and tissues relevant to areas of biosecurity. In another example, a complex biological sample or media which may be subjected to a method of detection of the disclosure is a food or beverage sample. In some of the examples disclosed herein, the complex biological sample or media may be subjected to minimal or no sample preparation procedures. An example in which the complex biological sample or media is subjected to minimal sample preparation is where a complex biological sample is simply diluted, dissolved or stabilised in an appropriate liquid for testing, such as water, saline or a buffer. In other examples, a complex biological sample or media which is subjected to minimal sample preparation is fractionated. Process steps performed on a complex biological sample may, in some examples, reduce complexity of the sample. However, in other examples, the complex biological sample may be unprocessed, such that the detection method of the disclosure is performed on the sample directly.

Detection of the nucleic acid analyte or biomarker may form part of a diagnosis of a disease, condition or injury in a subject, for example a brain injury such as TBI. Accordingly, the at least one nucleic acid analyte may be associated with a condition, disease state or injury. In one particular example, the at least one nucleic acid analyte is associated with a brain injury, optionally a traumatic brain injury (TBI), and the method is for diagnosing a brain injury (e.g., TBI). Other medical indications are described and contemplated herein. However, as described herein, the porous substrate, electrode, electrochemical cell and/or device is not limited to detecting nucleic acid analytes associated with brain injury exclusively, or even medical conditions for that matter. The porous substrate, electrode, electrochemical cell and/or device may equally be used to detect nucleic acids associated with biological source (e.g., injury, disease state, pathogen, contaminants etc), in other complex biological samples. This may occur via the detection of one or more nucleic acid analytes associated with the condition, injury, disease state, pathogen, contaminants etc, which may take various forms as described herein. As described, the at least one nucleic acid analyte may take various forms. Exemplary nucleic acids which may be detected using the method of the disclosure include DNA, messenger RNA, ribosomal RNA, a transfer RNA, a microRNA, a short

interfering RNA, and plasmid DNA. In one example, the nucleic acid analyte detected in the method is miRNA. In one particular example, the method of the disclosure comprises detecting the presence or absence of one or more miRNA associated with TBI in a complex biological sample (e.g., blood, a blood fraction or a diluted blood solution). For
5 example, the one or more miRNA may be selected from the group consisting of miR-142-3p, miR-150-5p, miR-196b-5p, let-7f-5p, miR-196b-5p and combination thereof.

In some examples, the method comprises determining the severity of a condition, disease state or injury by determining a concentration of at least one biomarker, for example at least one nucleic acid analyte, in a sample. In the case of disease or injury,
10 this may allow a user to determine the severity of a particular injury or disease, and possible differentiate between mild and severe cases. In the case of a pathogen or contaminant, it may enable a user to determine whether the analyte is present above or below a threshold level which is relevant to decision making. This analysis and the generation of results may be within a short timeframe, for example within 60, 40, 30, 20,
15 15 or 10 minutes.

The porous substrate, electrode, electrochemical cell and/or device, or method of the disclosure, may also be used in personalised medicine, for example, in determining the likelihood that a subject will contract a given disease or condition based on the presence, absence or concentration of one or more nucleic acid biomarkers; determining
20 the likelihood that a subject with a disease or condition will respond to therapy based on the presence, absence or concentration of one or more nucleic acid biomarkers; determining the prognosis of a subject with a disease or condition (or its likely progression or regression), based on the presence, absence or concentration of one or more nucleic acid biomarkers; and/or determining the effect of a treatment on a subject
25 with a disease or condition based on the presence, absence or concentration of one or more nucleic acid biomarkers. In some of the examples disclosed herein, the method of the disclosure is capable of detecting at least one nucleic acid analytes in a complex biological sample at a concentration of about 1 pM or less.

The use of porous substrate, electrode, electrochemical cell and/or device may allow for the manufacture of equipment that could be utilised on a benchtop, in a form
30 that is handheld, in a clinical setting, or out in the field, allowing for quick diagnoses, and the potential ability of obtaining results (which can dictate medical decisions), *in situ*. Training on this equipment may also allow for a broader range of individuals to be trained, with simpler training required in relation to other technologies, such as PCR
35 based detection.

Also disclosed herein is use of the porous substrate as described herein, the electrode as described herein, the electrochemical cell as described herein, and/or the device as described herein, to determine a concentration of at least one nucleic acid analyte in a complex biological sample. The nucleic acid analyte may be selected from, but not limited to: a messenger RNA (mRNA), ribosomal RNA (rRNA), a transfer RNA (tRNA), a microRNA (miRNA), a short interfering RNA (siRNA), and mixtures thereof. In one particular example the nucleic acid analyte is associated with a brain injury, such as a TBI. However, a skilled person will appreciate that nucleic acid analytes can be used as biomarkers for a range of injuries, conditions or diseases, including but not limited to, cancers, kidney disease (including acute kidney injury), cardiovascular disease, autoimmune diseases, infectious diseases, metabolic disease, liver disease and diseases of the blood. Thus, the porous substrate, the electrode, the electrochemical cell, and/or device as described herein may equally be used to determine a concentration of at least one nucleic acid analyte associated with any one of the aforementioned conditions in a sample. Furthermore, the porous substrate, the electrode, the electrochemical cell, and/or device as described herein may equally be used to determine a concentration of at least one nucleic acid analyte associated with pathogens and/or contaminants of interest. Suitable pathogens are described hereinabove and shall apply *mutatis mutandis* to this example. In some of the foregoing example, the concentration of the nucleic acid analyte may be used to make a diagnostic decision, e.g., determine the severity of a disease state or condition in a subject, or determine a level of contaminant etc. In one example, the concentration of the one or more nucleic acid analytes associated with brain injury (e.g., TBI) is used to determine the severity of the brain injury (e.g., TBI).

In one embodiment, the porous substrate, the electrode, the electrochemical cell, and/or device as described herein is used to detect at least one nucleic acid analyte in a liquid, complex biological sample. Exemplary complex biological samples are described herein. In one example, the liquid sample is a bodily fluid. In one particular example, the complex biological sample is blood or a blood fraction (e.g., serum).

Also disclosed herein is a method of detecting at least one nucleic acid analyte in a sample, the method comprising introducing the sample (e.g., a liquid sample), to an electrochemical biosensor comprising: the porous substrate as described herein, the electrode as described herein, the electrochemical cell as described herein, and/or the device as described herein, and determining the presence or absence of the nucleic acid analyte in the sample.

Also disclosed herein is a method of detecting at least one nucleic acid analyte in a sample, the method comprising introducing the sample (e.g., a liquid sample), to the porous substrate as described herein, the electrode as described herein, the electrochemical cell as described herein, and/or the device as described herein, and determining the presence or absence of the at least one nucleic acid analyte in the sample. In one embodiment this is based on measurements of peak current obtained from differential pulse voltammograms prior to and after introducing the sample to the electrochemical biosensor, wherein detection of a change in the peak current after introduction of the sample to the electrochemical biosensor indicates the presence of the nucleic acid analyte in the sample and detection of no change in the peak current after introduction of the sample to the electrochemical biosensor indicates that the nucleic acid analyte is not present in the sample above a threshold level. An advantage of detecting nucleic acid analytes using the porous substrate, the electrochemical biosensor, the electrochemical cell, and/or the device as described herein, is the ability to detect the nucleic acid in complex biological samples or mediums as described herein. In this regard, the control and selection of the dimensions of the plurality of channels in the porous substrate may negate or reduce problems associated with biofouling. In this regard, for sensors deployed and/or exposed to biological samples, the signal from any channel blockage can be influenced by biofouling mechanisms, for example where foulants attach to a portion of an electrode (for example an electrode derived from or based on a porous substrate as described herein), via non-specific adsorption. The foulants can potentially reduce the area of electrode surface that is available to one or more analytes, causing a decrease, for example, of a differential pulse voltammetry signal. In the case of the present disclosure, the control and selection of the dimensions of the plurality of channels may negate or reduce problems associated with biofouling. This may be due, at least in part, to the dimensions of the channel being on a scale such that proteins and/or other potential biomolecules that give rise to fouling (such as those that may be present in complex biological samples comprising the analytes of interest), cannot enter or progress through a channel, whilst the analytes of interest are on a scale that means they are sufficiently small and mobile to reach capture probes disposed within the plurality of channels. Thus, a potential advantage of the method of the disclosure is that it can be capable of detecting nucleic acid analytes rapidly in complex biological samples.

The method of determining the change in peak current may be performed using any appropriate method in the art. For example, the change in the peak current may be normalised via the relationship of Equation 1:

$$\Delta I = (I_0 - I_n)/I_0 \quad (1)$$

where ΔI is a normalised current change, I_0 is a peak current value measured after incubation for a first period of time in a buffer, and I_n is the peak current value measured after incubation for a second period in a target solution at a given concentration, in comparison to a control sample. Herein the first period of time and/or the second period of time may be independently about or less than about: 60 minutes, 50 minutes, 40 minutes, 30 minutes or 20 minutes of contacting the sample. For example the detection of the at least one analyte may be possible within about: 1 minute, 2 minutes, 3 minutes, 4 minutes, 5 minutes, 6 minutes, 7 minutes, 8 minutes, 9 minutes, 10 minutes, 11 minutes, 12 minutes, 13 minutes, 14 minutes, 15 minutes, 16 minutes, 17 minutes, 18 minutes, 19 minutes, 20 minutes, 21 minutes, 22 minutes, 23 minutes, 24 minutes, 25 minutes, 26 minutes, 27 minutes, 28 minutes, 29 minutes, 30 minutes, 35 minutes, 40 minutes, 45 minutes, 50 minutes, 55 minutes, or about 60 minutes.

The method may comprise determining the concentration of the nucleic acid analyte detected in the sample based on a level of reduction in peak current intensity. The method may further comprise comparing the concentration of the at least one nucleic acid analyte with a calibration curve.

In some examples, one or more nucleic acid analytes are detected, and concentration optionally quantified, within about: 60 minutes or less, 30 minutes or less, or 20 minutes or less of contacting the sample. For example the detection of the at least one analyte may be possible within about: 1 minute, 2 minutes, 3 minutes, 4 minutes, 5 minutes, 6 minutes, 7 minutes, 8 minutes, 9 minutes, 10 minutes, 11 minutes, 12 minutes, 13 minutes, 14 minutes, 15 minutes, 16 minutes, 17 minutes, 18 minutes, 19 minutes, 20 minutes, 21 minutes, 22 minutes, 23 minutes, 24 minutes, 25 minutes, 26 minutes, 27 minutes, 28 minutes, 29 minutes, 30 minutes, 35 minutes, 40 minutes, 45 minutes, 50 minutes, 55 minutes, or about 60 minutes.

EXAMPLE EMBODIMENTS

The following are Example Embodiments according to the various aspects described herein:

1. A method of detecting at least one nucleic acid analyte in a complex biological sample, the method comprising:
 - (i) introducing the sample to an electrochemical biosensor comprising a porous substrate comprising a plurality of channels, wherein:

- at least a portion of the plurality of channels has a mean depth of about 1.0 μm to about 6.0 μm ; and
 - at least a portion of the plurality of channels has a mean diameter or cross section in a range of about 15 nm to about 40 nm,
- 5 wherein one or more capture probes capable of binding specifically to at least biomarker, optionally at least one nucleic acid analyte, to form a probe-analyte complex, are disposed on at least one surface of at least a portion of the plurality of channels; and
- (ii) determining the presence or absence of the at least one nucleic acid analyte in the sample based on measurements of peak current obtained from differential pulse
- 10 voltammograms prior to and after introducing the sample to the electrochemical biosensor,
- wherein detection of a change in the peak current after introduction of the sample to the electrochemical biosensor indicates the presence of the at least one nucleic acid analyte in the sample and detection of no change in the peak current after introduction of
- 15 the sample to the electrochemical biosensor indicates that the at least one nucleic acid analyte is not present in the sample above a threshold level.
2. The method according to example embodiment 1, wherein the capture probe is capable of binding specifically to at least one nucleic acid analyte to form a probe-analyte
- 20 complex, wherein the presence of the probe-analyte complex within a channel causes at least a partial steric and/or charge blockage to the channel as compared to the channel in the absence of the probe-analyte complex.
3. The method according to any one of example embodiments 1 to 3, wherein:
- 25
- at least 10% of the plurality of channels in the porous substrate have a mean depth in a range of about 1.0 μm to about 2.0 μm , or about 1.0 μm to about 4.0 μm ; and/or
 - at least 10% of the plurality of channels in the porous substrate have a mean diameter or cross section in a range of about 15 nm to about 30 nm, or about
- 30 20 to about 30 nm.
4. The method according to any one of example embodiments 1 to 3, wherein:
- the porous substrate comprises, consists essentially of, or consists of, a conducting material or semi-conducting material; and/or
- 35
- the porous substrate comprises, consists essentially of, or consists of, a material selected from the group consisting of: platinum, gold, silver,

- cobalt, gallium, indium, tin, vanadium, zirconium, copper, aluminium, iridium, palladium, rhodium, silicon, zinc, iron, steel, brass, carbon, graphite, alloys, oxides, or metallic compounds of these elements, and mixtures thereof; and/or
- 5 - the porous substrate is not in the form of a film; and/or
- the porous substrate does not comprise nanoparticles and/or is composed of nanoparticles; and/or
- the porous substrate does not comprise one or more of aluminium, platinum gold, and/or silver; and/or
- 10 - the porous substrate comprises, consists essentially or consists of a material selected from silicon, optionally a heat treated and/or chemically modified silicon; and/or
- a layer comprising, consisting essentially thereof, or consisting of, carbon is disposed on at least a portion of the porous substrate.
- 15
5. The method according to any one of example embodiments 1 to 4, wherein:
- the porous substrate, optionally comprising silicon, comprises, or consists essentially of, or consists of, a single layer comprising one or more of:
- 20 ▪ one or more capture probes capable of binding specifically to at least one biomarker, optionally at least one nucleic acid analyte, to form a probe-analyte complex; and/or
- one or more functional groups capable of reacting with an amine group; and/or
- 25 ▪ one or more functional groups capable of forming a covalent bond with a complementary functional groups on one or more capture probes that are capable of binding specifically to at least one biomarker, optionally at least one nucleic acid analyte, to form a probe-analyte complex; and/or
- one or more carboxylic acid groups; or
- 30 - the porous substrate, optionally comprising silicon, comprises, consists essentially of or consists essentially of or consists of at least one layer comprising one or more of:
- one or more capture probes capable of binding specifically to at least one biomarker, optionally at least one nucleic acid analyte, to form a probe-analyte complex; and/or
- 35

- 5
- one or more functional groups capable of reacting with an amine group; and/or
 - one or more functional groups capable of forming a covalent bond with a complementary functional groups on one or more capture probes that are capable of binding specifically to at least one biomarker, optionally at least one nucleic acid analyte, to form a probe-analyte complex; and/or
 - one or more carboxylic acid groups.
- 10 6. The method according to any one of example embodiments 1 to 5, wherein:
- the one or more capture probes are capable of binding at a plurality of different nucleic acid analytes; and/or
 - the at least one nucleic acid analyte is selected from the group consisting of: a DNA analyte, a messenger RNA (mRNA) analyte, ribosomal RNA (rRNA) analyte, a transfer RNA (tRNA) analyte, a microRNA (miRNA) analyte, a short interfering RNA (siRNA) analyte, plasmid DNA (pDNA) and mixtures thereof; and/or
 - one or more of the at least one capture probe is a DNA capture probe or a peptide nucleic acid probe (PNA) probe.
- 15 20
7. The method according to any one of example embodiments 1 to 6, wherein:
- the at least one nucleic acid analyte is associated with a condition, disease state or injury; and/or
 - the at least one nucleic acid analyte is associated with a brain injury, optionally a traumatic brain injury.
- 25
8. The method according to any one of example embodiments 1 to 7, wherein the at least one nucleic acid analyte is a miRNA associated with a brain injury, optionally a traumatic brain injury.
- 30
9. The method according to example embodiment 6 or example embodiment 8, wherein the one or more miRNAs are selected from the group consisting of miR-142-3p, miR-150-5p, miR-196b-5p, let-7f-5p, or any combination thereof

10. The method according to any one of example embodiments 1 to 9, wherein the at least one nucleic acid analytes is detected in the sample at a concentration of about 1 pM or less.
- 5 11. The method according to any one of example embodiments 1 to 10, wherein the complex biological sample is or comprises a body fluid, cell culture medium, water sample, a soil sample, food or beverage sample, an agricultural product, surface debris, a surface swab or any combination thereof.
- 10 12. The method according to any one of example embodiments 1 to 11, wherein the complex biological sample is:
- from a human; and/or
 - a liquid sample; and/or
 - selected from the group consisting of: whole blood, serum, plasma, urine,

15 saliva, sweat, spinal fluid, sputum, cell lysate, cell culture medium and mixtures thereof; and/or

 - obtained from a subject suspected of having suffered a brain injury, optionally a traumatic brain injury.
- 20 13. The method of any one of example embodiments 1 to 12, wherein the electrochemical biosensor comprises one or more electrodes.
14. The method according to example embodiment 13, wherein the electrochemical biosensor comprises:
- 25
- a working electrode comprising the porous substrate;
 - a reference electrode; and
 - a counter electrode.
15. The method according to example embodiment 13 or example embodiment 14,
- 30 wherein an insulating material is disposed on at least a portion of the electrodes.
16. The method according to any one of example embodiments 1 to 15, wherein the electrochemical biosensor is used in conjunction with (i) a device capable of scanning a potential within a certain range and measure current or (ii) a potentiostat.

17. The method according to any one of example embodiments 1 to 16, wherein a change in the peak current is normalised via the relationship of Equation 1:

$$\Delta I = (I_0 - I_n)/I_0 \quad (1)$$

5 where ΔI is a normalised current change, I_0 is a peak current value measured after incubation for a first period of time in a buffer, and I_n is the peak current value measured after incubation for a second period in a target solution at a given concentration, in comparison to a control sample.

18. The method according to example embodiment 17, wherein the first period of time and/or the second period of time is independently about or less than about: 60 minutes, 50 minutes, 40 minutes, 30 minutes or 20 minutes.

19. The method according to any one of example embodiments 1 to 18, comprising:

- 15 - determining the concentration of the nucleic acid analyte detected in the sample based on a level of reduction in peak current intensity;
- optionally comparing the concentration of the at least one nucleic acid analyte with a calibration curve; and
- optionally determining the severity of a disease state or injury, optionally a brain injury in a subject based on the concentration of the at least one nucleic acid analyte.

20

20. The method according to any one of example embodiments 1 to 19, wherein the at least one nucleic acid analyte is detected, and concentration optionally quantified, within: about 60 minutes, about 50 minutes, about 40 minutes, about 30 minutes, about 25 20 minutes, about 15 minutes or about 10 minutes, of introducing the sample to the electrochemical biosensor.

21 A porous substrate when used in a method according to any one of example embodiments 1 to 20, said porous substrate comprising a plurality of channels, wherein:

- 30 - at least a portion of the plurality of channels has a mean depth of about 1.0 μm to about 6.0 μm ; and/or
- at least a portion of the plurality of channels has a mean diameter or cross section in a range of about 15 nm to about 40 nm,

wherein one or more capture probes capable of binding specifically to at least one nucleic acid analyte to form a probe-analyte complex are disposed on at least one surface of at least a portion of the plurality of channels.

35

22. The porous substrate when used according to example embodiment 21, wherein the capture probe is capable of binding specifically to at least one nucleic acid analyte to form a probe-analyte complex, wherein the presence of the probe-analyte complex within a channel causes at least a partial steric and/or charge blockage to the channel as compared to the channel in the absence of the probe-analyte complex.
23. The porous substrate when used according to example embodiment 21 or example embodiment 22 wherein:
- at least a portion of the plurality of channels has a mean depth of about 1.0 μm to about 6.0 μm ; and
 - at least a portion of the plurality of channels has a mean diameter or cross section in a range of about 15 nm to about 40 nm.
24. The porous substrate when used according to any one of example embodiments 21 to 23, wherein:
- at least 10% of the plurality of channels have a mean depth in a range of about 1.0 μm to about 6.0 μm ; and/or
 - at least 10% of the plurality of channels have a mean diameter or cross section in a range of about 15 nm to about 40 nm.
25. The porous substrate when used according to any one of example embodiments 21 to 24, wherein:
- the porous substrate comprises, consists essentially of, or consists of, a conducting or semi-conducting material; and/or
 - the porous substrate comprises, consists essentially of, or consists of, a material selected from the group consisting of: platinum, gold, silver, cobalt, gallium, indium, tin, vanadium, zirconium, copper, aluminium, iridium, palladium, rhodium, silicon, zinc, iron, steel, brass, carbon, graphite, alloys, oxides, or metallic compounds of these elements, and mixtures thereof; and/or
 - the porous substrate comprises, consists essentially or consists of a material selected from silicon; and/or
 - a layer comprising, consisting essentially thereof, or consisting of carbon is disposed on at least a portion of the porous substrate.

26. The porous substrate when used according to any one of example embodiments 21 to 25, wherein:

- the porous substrate, optionally comprising silicon, comprises, or consists essentially of, or consists of, a single layer comprising one or more of:
 - 5 ▪ one or more capture probes capable of binding specifically to at least one biomarker, optionally at least one nucleic acid analyte, to form a probe-analyte complex; and/or
 - 10 ▪ one or more functional groups capable of reacting with an amine group; and/or
 - 15 ▪ one or more functional groups capable of forming a covalent bond with a complementary functional groups on one or more capture probes that are capable of binding specifically to at least one biomarker, optionally at least one nucleic acid analyte, to form a probe-analyte complex; and/or
 - 20 ▪ one or more carboxylic acid groups; or
- the porous substrate, optionally comprising silicon, comprises, consists essentially of or consists essentially of or consists of at least one layer comprising one or more of:
 - 25 ▪ one or more capture probes capable of binding specifically to at least one biomarker, optionally at least one nucleic acid analyte, to form a probe-analyte complex; and/or
 - 30 ▪ one or more functional groups capable of reacting with an amine group; and/or
 - 35 ▪ one or more functional groups capable of forming a covalent bond with a complementary functional groups on one or more capture probes that are capable of binding specifically to at least one biomarker, optionally at least one nucleic acid analyte, to form a probe-analyte complex; and/or
 - one or more carboxylic acid groups.

27. The porous substrate when used according to any one of example embodiments 21 to 26, wherein:

- the one or more capture probes are capable of binding at a plurality of different nucleic acid analytes; and/or
- 35 - the at least one nucleic acid analyte is selected from the group consisting of: a DNA analyte, a messenger RNA (mRNA) analyte, ribosomal RNA

- (rRNA) analyte, a transfer RNA (tRNA) analyte, a microRNA (miRNA) analyte, a short interfering RNA (siRNA) analyte, plasmid DNA (pDNA) and mixtures thereof; and/or
- one or more of the at least one capture probe is a DNA capture probe or a peptide nucleic acid probe (PNA) probe.
28. The porous when used substrate according to any one of example embodiments 21 to 27, wherein:
- the at least one nucleic acid analyte is associated with a condition, disease state or injury; and/or
 - the at least one nucleic acid analyte is associated with a brain injury, optionally a traumatic brain injury.
29. The porous substrate when used according to any one of example embodiments 21 to 28, wherein the at least one nucleic acid analyte is a miRNA associated with a brain injury, optionally a traumatic brain injury.
30. The porous substrate when used according to example embodiment 29, wherein the one or more capture probes are capable of binding one or more miRNAs selected from the group consisting of miR-142-3p, miR-196b-5p, let-7f-5p, miR-150-5p and combinations thereof.

EXAMPLES

Materials

P-type Si wafers with 0.00055–0.001 Ω cm resistivity, (100)-oriented were purchased from Siltronix (France). Hydrofluoric acid (HF) (48%, AR grade) was purchased from Scharlau (Australia). Potassium ferrocyanide ($K_4[Fe(CN)_6]$), potassium ferricyanide ($K_3[Fe(CN)_6]$), undecylenic acid, *N*-hydroxysuccinimide (NHS), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC), phosphate-buffered saline (PBS) tablets, 2-(*N*-morpholino)-ethanesulfonic acid (MES), sodium chloride (NaCl), sodium hydroxide, ethanolamine, and tris(hydroxymethyl)aminomethane were purchased from Sigma-Aldrich (Australia). The acetylene gas cylinder was purchased from BOC (Australia). All DNA and miRNA strands were purchased from Integrated DNA Technologies, Inc. The sequences of capture ssDNA, target ssDNA and target miRNA are all listed in Table 1.

Table 1. Sequences of ssDNA capture probes, ssDNA targets, and miRNA targets.

Custom Oligomers	Channel Depth (μm)
miR-142-ssDNA-capture	5'-/5AmMC6/TC CAT AAA GTA GGA AAC ACT ACA-3'
miR-142-ssDNA-target	3'-TGT AGT GTT TCC TAC TTT ATG GA-5'
miR-142-RNA-target	3' rUrGrT rArGrU rGrUrU rUrCrC rUrArC rUrUrU rArUrG rGrA-5'
miR-423-ssDNA-capture	5'-/5AmMC6/AC TGA GGG GCC TCA GAC CGA GCT-3'

Fabrication of porous silicon

5 A whole 6 inch p-type Si wafer was anodically etched in an electrolyte solution containing 1:1 (v:v) HF and absolute ethanol to produce a first pSi layer, using an MPSB wet etching system (AMMT GmbH). Firstly, a sacrificial layer was produced at an anodic current of 1 A for 90 s. This was removed with 1 M sodium hydroxide. Then the etching cell was rinsed with water, absolute ethanol, and dried with N₂ gas. This step aids
10 by ideally inhibiting the formation of a parasitic layer during further etching of a proper pSi sensing layer. Next, pSi samples with varying channel size and thickness/diameters were fabricated in 1:1 etchant by changing the current density and time to study the effect of the morphological features on their electrochemical performance (Table 2). The freshly etched pSi was rinsed with ethanol and then immediately used for carbon
15 stabilisation.

Table 2. Etching conditions for fabricating pSi with different parameters.

Channel size (nm)	Channel depth (μm)	Current (A)	Etching time (s)
27	1.0	1	272
27	1.7	1	463
27	5.7	1	1300
15	1.7	0.55	633
40	1.7	5	100

Thermal hydrocarbonisation treatment of freshly etched porous silicon

To improve the stability and conductivity of pSi single layer nanostructures, the freshly etched pSi was placed into a quartz tube under N₂ flow at 2 L·min⁻¹ for 45 minutes at room temperature. A 1:1 N₂-acetylene mixture flow was introduced into the tube at room temperature for 15 minutes after the purging step, then the quartz tube was placed into a preheated tube furnace at 525 °C for another 15 minutes under the continuous mixture flow. Finally, the tube was allowed to cool back to room temperature under the N₂ flow, yielding THCpSi samples with a THCpSi layer.

Surface functionalisation and immobilisation of capture probes

Firstly, in order to functionalise the THCpSi layer with -COOH groups, the sample was immersed into pure undecylenic acid at 150 °C for 10 hours under an inert atmosphere (N₂). After cooling down to room temperature, the sample was rinsed with absolute ethanol. The -COOH groups at the THCpSi layer were activated by incubating the functionalised substrates in 10 mg·mL⁻¹ 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), and 15 mg·mL⁻¹ N-hydroxysuccinimide (NHS), in 0.1 M MES buffer, pH 5.5, at room temperature for 30 minutes to produce succinimidyl ester groups. Subsequently, 200 µL of either 10 µM of NH₂-ssDNA capture probe or nonspecific NH₂-ssDNA capture probe, both prepared in 10 mM PBS, were incubated on the activated surface overnight at 4 °C. Samples were subsequently rinsed thoroughly with 10 mM PBS followed by the addition of 200 µL of 0.1 M ethanolamine in 10 mM PBS to the cell for 45 minutes incubation at room temperature to quench the unreacted succinimidyl ester group. Finally, samples were washed again with 10 mM PBS solution and ready to proceed with sensing experiments.

Scanning electron microscopy (SEM)

To characterise the morphology of the biosensor surface, samples were mounted on an aluminium stub using double-sided conductive carbon tape. SEM images were obtained with an FEI NovaNano SEM 430 at accelerating voltages ranging from 10 kV to 19 kV.

Fourier transform infrared spectroscopy (FTIR)

IR spectra were recorded on a Thermo Scientific Nicolet 6700 FT-IR spectrometer using reflectance mode. Boron doped (p-type) Si, (100)-orientated with 1.1–1.8 Ω cm and 0.00055–0.001 Ω cm resistivity was used for IR transmission and reflectance measurements, respectively. All the spectra were recorded as an average of 64 scans at a

resolution of 8 cm^{-1} over the range of 650 to 4000 cm^{-1} . A clean flat Si substrate was used as background.

Water contact angle measurements

5 A custom-built goniometer with a Panasonic CCTV camera (WV-BP550/G) was used to conduct water contact measurements of the pSi structures. After dropping $1\text{ }\mu\text{L}$ ultrapure water onto the surface with a $10\text{ }\mu\text{L}$ syringe, a photograph was immediately taken. ImageJ software (Drop Analysis plugin), was employed to determine the contact angle of the water drop in contact with the surface.

10

Electrochemical characterisation

All the electrochemical measurements were performed on an electrochemical analyser (Metrohm Autolab potentiostat), using a three-electrode electrochemical cell. A custom-designed Teflon cell was assembled to host the modified THCpSi slide as the
15 working electrode, silver/silver chloride was used as the reference electrode, and a platinum wire was used as the counter electrode. Data acquisition and analysis were accomplished using Metrohm Nova 2.1 software. All the potentials were provided versus Ag/AgCl. Cyclic voltametric (CV) measurements were performed by scanning the potential in a potential range that depends on the redox species used, at a scan rate of 0.1
20 V s^{-1} . Electrochemical impedance spectroscopy (EIS) measurements were performed under open circuit potential conditions, scanning frequencies from 100 kHz to 0.1 Hz in logarithmic scale, with an AC amplitude of 5 mV . Differential pulse voltammetry (DPV), measurements were acquired by scanning the potential from -0.2 to 0.6 V .

25 DNA and miRNA detection using THCpSi-based sensor

To assess the performance of the developed miRNA sensors, ssDNA and miRNA target solutions, prepared at various concentrations (from 0.1 to 1000 pM), in 10 mM Tris buffer with 75 mM NaCl , $\text{pH } 7.5$, respectively, were incubated on the sensor surface for 15 minutes. After each incubation step, the biosensor surfaces were thoroughly
30 washed with PBS and transferred to a $2\text{ mM } [\text{Fe}(\text{CN})_6]^{3-/4-}$ solution in 10 mM PBS . In order to detect miRNA biomarkers in diluted serum, fetal bovine serum (FBS) was added to the above Tris buffer solution to obtain 2% final concentration of FBS, and miRNA target solutions at various concentrations (from 0.1 to 1000 pM) were prepared in 2% FBS/Tris buffer. To verify the current changes were only caused from the specific
35 hybridisation between the immobilised ssDNA capture probe on the carbon-stabilised pSi surface and the incubated target ssDNA, the same measurements were repeated using

control biosensors prepared under identical conditions but using random sequences for the ssDNA capture probe. Human serum samples were collected from human healthy donors and mTBI patients from the Alfred Hospital, Melbourne, Australia. The collected serum samples were then diluted to 2% concentration by the same Tris buffer solution.

5

Example 1 – Fabrication and thermal hydrocarbonisation of pSi

Five samples representing pSi were fabricated by anodically etching p-type Si wafers in a wet etching system according to the aforementioned procedure. The channel size and depth was easily controlled and adjusted by tuning the anodisation conditions, including current density and etching time. To stabilise the pSi surface and increase the electrical conductivity of the semiconductor, freshly etched pSi samples were subjected to a thermal treatment at 525 °C.

The SEM images in Figure 1 show the top (Figure 1a, c, e, g and i), and cross-sectional (Figure 1b, d, f, h, j), features of the five THCPsi samples with various channel sizes and thicknesses. Under different etching conditions, the average channel diameter can be modified from 15 nm to 50 nm, while the channel depth ranged from 1.0 µm to 5.7 µm.

Moreover, water contact angle measurements provided evidence that the surface chemistry of pSi has been changed after thermal hydrocarbonisation (Figure 2). The significantly larger contact angle of THCPsi substrates (Figure 2b), compared to that of pSi substrates (Figure 2a), with the same dimensions clearly demonstrates the hydrophobic and hydrophilic behaviour of THCPsi and pSi, respectively, which is expected from thermally hydrocarbonised coatings and saturated hydrocarbon chains.

Example 2 - Surface functionalities on THCPsi

The surface of a THCPsi sample was modified according to the above procedure. The surface modification steps included the introduction of carboxylic groups and covalent attachment of the bioreceptors.

FTIR was used to characterise the functionalisation of THCPsi with carboxylic groups. Figure 3 shows the FTIR spectra collected from freshly etched pSi, THCPsi, and COOH-terminated THCPsi.

As expected, freshly etched pSi displays bands characteristic of Si-H and Si-H₂ stretching vibrations at 2087 and 2114 cm⁻¹ with an additional band assigned to the Si-H deformation at 905 cm⁻¹ (line A). After generating a hydrocarbonised layer throughout the surface, the bands characteristic of Si-H disappeared in THCPsi, but a new peak

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associated with CH_3 symmetric deformation of $\text{Si}-\text{CH}_3$ appeared at $1250-1260\text{ cm}^{-1}$ (line B). Furthermore, two prominent absorption bands became evident at 2852 and 2920 cm^{-1} , which can be assigned to the symmetric and asymmetric CH_2 stretching modes, respectively. Followed by thermal treatment, the surface of THCpSi was thermally hydrosilylated with a COOH -terminated alkene (10-undecanoic acid), which was in turn used to attach the NH_2 -ssDNA capture probe as a bioreceptor within the nanochannels. Here, the miR-142-ssDNA capture probe was used to fabricate the biosensor. A peak at 1720 cm^{-1} was observed in the FTIR spectra prior to attachment of the bioreceptor, which is attributed to the carbonyl stretching vibration (line C). These results reveal the successful changes of the chemical composition during surface functionalisation.

Example 3 - Electrochemical characterisations of THCpSi electrodes

The electrochemical properties of the THCpSi nanostructure were first investigated by cyclic voltammetry (CV) in the presence of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 10 mM phosphate-buffered saline solution (PBS), at $\text{pH } 7.4$. The evolution of oxidation and reduction reactions were monitored by observing the current intensity change, as well as the shift of redox potentials, when a cyclic potential is applied between the working and reference electrodes.

Figure 4a depicts the electrochemical characterisation of THCpSi , as cyclic voltammograms (CVs) of freshly etched pSi (line 1), THCpSi (line 2), COOH -terminated THCpSi (line 3), and ssDNA-modified THCpSi (line 4) electrodes. Figure 4b depicts the electrochemical impedance spectroscopy (EIS) spectra of THCpSi (line 1), COOH -terminated THCpSi (line 2), and ssDNA-modified THCpSi (line 3) electrodes. Measurements were performed in a 2 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ solution in 10 mM PBS, $\text{pH } 7.4$.

As shown in Figure 4a, the low oxidation and reduction peak currents indicate the poor performance of freshly etched pSi as electrochemical transducer (Figure 4a, line 1). THCpSi (Figure 4a, line 2), shows fast and reversible electrochemical response to $[\text{Fe}(\text{CN})_6]^{3-/4-}$, representing a fully reversible one-electron transfer reaction. The results suggest that THCpSi possesses the required surface structure and electronic properties to enable rapid electron transfer. Prior to the immobilisation of the ssDNA capture probe, the COOH -terminated THCpSi surface was activated by EDC/NHS reaction to generate succinimidyl ester groups, which are known to react with available primary amino groups on ssDNA. Both COOH -terminated THCpSi and ssDNA-modified THCpSi were electrochemically characterised by CV measurements. The COOH -terminated THCpSi

features a larger peak separation and lower peak (Figure 4a, line 3) current than those of bare THCpSi, while these features become more prominent in the ssDNA-modified THCpSi (Figure 4a, line 4).

The electron transfer processes through the interface between THCpSi electrode and $[\text{Fe}(\text{CN})_6]^{3-/4-}$ were further explored by use of electrochemical impedance spectroscopy (EIS). Impedance measurements were performed at the open circuit potential for a range of frequencies (100 kHz – 0.1 Hz) and 5 mV amplitude. The impedance spectra were fitted to a Randles circuit, which is an equivalent electrical circuit composed of solution resistance (R_s), charge transfer resistance (R_{ct}), Warburg impedance (W), and double layer capacitance (C_{dl}). It is worth noting that the bare THCpSi displays a much smaller R_{ct} value, as indicated by a straight line in Figure 4b (line 1), demonstrating the high conductivity of carbon-stabilised pSi nanostructures as electrochemical transducers. After surface functionalisation, impedance spectra showed a significant increase in R_{ct} of both COOH-terminated THCpSi (Figure 4b, line 2) and ssDNA-modified THCpSi (Figure 4b, line 3). Therefore, these results confirm that the ssDNA capture probe was present on the surface after surface conjugation, demonstrating the feasibility of using these carbon-stabilised pSi nanostructures as promising electrochemical transducers. Furthermore, repeatability and reproducibility for THCpSi platforms were assessed by analysing both CV and EIS data, showing excellent repeatability.

Example 4 - Optimising the parameters of THCpSi

In contrast to conventional carbon-based electrodes, such as glassy carbon electrode and carbon screen-printed electrode, the electrode surface area of THCpSi can be easily tuned by changing the etching conditions. As a result, one of the main advantages of this carbon-stabilised pSi over conventional carbon electrodes is that the sensitivity of the sensing platform can be improved by adjusting the channel dimensions to the desired application. To demonstrate the suitability of the THCpSi nanostructures for electrochemical biosensing, a label-free voltammetric miRNA biosensor was constructed.

The sensing mechanism of the label-free voltammetric miRNA sensor developed here relies on the hypothesis that ssDNA - miRNA hybridisation will induce partial steric or charge-based blockage of the nanochannels. When the target miRNA hybridises with the ssDNA capture probe immobilised at the THCpSi layer, partial blockage of the porous nanochannels can be expected. This partial nanochannel blockage hinders the

diffusion of redox species, such as $[\text{Fe}(\text{CN})_6]^{3-/4-}$, into the electrochemically active layer (the electrochemical transducer), resulting in a decrease in the intensity of peak current monitored by DPV measurements. Changes in peak current obtained from differential pulse voltammograms acquired prior to and after hybridisation were normalised via the relationship of Equation 1:

$$\Delta I = (I_0 - I_n)/I_0 \quad (1)$$

where ΔI is the normalised current change, I_0 is the peak current value measured after 20 minutes incubation in buffer blank, and I_n is the peak current value measured after 20 minutes incubation in the miRNA target solution at a given concentration. Control samples were all prepared using a random sequence of ssDNA as a capture probe (miR-423-ssDNA-capture).

In order to develop a rapid, reliable, and highly sensitive electrochemical biosensor for miRNA biomarker detection, the essential parameters of the THCpSi platform including channel size and depth, need to be optimised. The average channel size of 27 nm was first kept the same, while the porous thickness was tuned from 1.0 μm to 5.7 μm , as shown in Figure 5a-f. As ssDNA is much more stable than the similar miRNA in buffer, the miR-142-ssDNA-target with analogous sequence to miR-142-miRNA biomarker was chosen in the optimisation processes. Biosensors were consecutively incubated with various concentrations of miR-142-ssDNA-target titre diluted with Tris buffer (as disclosed previously herein) from the lowest to the highest. All measurements were done in triplicate to verify the reproducibility.

Figure 5 depicts the performance of each DNA sensor as differential pulse voltammograms (DPVs) of control electrodes fabricated by THCpSi with: (a) 1.0 μm thickness; and (d) 1.7 μm thickness, upon consecutive incubations in target ssDNA solutions. The DPVs of the working electrodes fabricated by THCpSi with (b) 1.0 μm thickness; and (e) 1.7 μm thickness, upon consecutive incubations in target ssDNA solutions. The dosage response curves of the biosensors fabricated by THCpSi with: (c) 1.0 μm thickness; and (f) 1.7 μm thickness, incubated with consecutive dilutions of target ssDNA.

As illustrated in Figure 5, the THCpSi control electrodes with both average channel depths of 1.0 μm and 1.7 μm , where mismatched ssDNA probe (miR-423-ssDNA-capture) was immobilised, did not show any significant signal changes in their differential pulse voltammograms when spiked in target ssDNA buffer solutions with

various concentrations from 0.1 pM to 1000 pM (Figure 5a and d), demonstrating promising specificity of the biosensor. However, the current intensity decreased in both working electrodes, which were immobilised by miR-142-ssDNA-capture, with the increasing concentration of the target ssDNA, as shown in Figure 5b and e, indicating that the current changes observed were a result of specific binding between capture probe and target ssDNA in the nanochannels. The electrochemical sensing performance was compared by determining the sensitivity of the sensors calculated as the slope of the linear curve fit. Biosensors featuring 1.7 μm -thick transducers can provide a sensitivity of 0.05 pM^{-1} compared to the 1.0 μm -thick transducers.

After determining an appropriate thickness of THCPsi for the specific DNA sequence, the biosensors with different average channel sizes of 15 nm and 40 nm were further evaluated by the same manner.

Figure 6 depicts the performance of each DNA sensor as DPVs of control electrodes fabricated by THCPsi with: (a) average 15 nm porous diameter; and (d) 40 nm porous diameter, upon consecutive incubations in target ssDNA solutions. The DPVs of working electrodes fabricated by THCPsi with: (b) average 15 nm porous diameter; and (e) 40 nm porous diameter, upon consecutive incubations in target ssDNA solutions. The dosage response curves of the biosensors fabricated by THCPsi with: (c) average 15 nm porous diameter; and (f) 40 nm porous diameter, incubated with consecutive dilutions of target ssDNA.

As illustrated in Figure 6, both control electrodes with these two average channel diameters display the almost identical current intensities in their differential pulse voltammograms when spiked in target ssDNA buffer solutions with various concentrations (Figure 6a and d), while the current intensity decreased in both working electrodes with the increasing concentration of the target ssDNA, as shown in Figure 6b and e. It is noted that both biosensors exhibited similar an electrochemical sensitivity of 0.02 pM^{-1} when detecting the target ssDNA, slightly lower than that of biosensors with 27 nm channel size and 1.7 μm depth. These results reveal that the suitable nanochannel parameters of THCPsi based biosensor have been optimised for developing target miRNA sensing platforms.

Example 5 – Electrochemical detection of target miRNA

The TBI related miRNA (miR-142-3p) was chosen as a biomarker to test the sensing performance of the optimised THCPsi-based nanochannel blockage biosensor in both buffer and diluted serum. Figure 7a-c display the sensing results of miR-142-

miRNA-target with different concentrations in Tris buffer. Similar to the electrochemical detection of analogues ssDNA, the current intensity of DPVs shows significant decrease in the working electrodes, while it remains almost the same in the control electrodes when increasing the concentration of target miRNA from 0.1 pM to 1000 pM. The relevant large sensitivity of 0.04 pM^{-1} confirms the excellent sensing performance of the platform for a TBI related miRNA biomarker.

Typically, the signal of the channel blockage electrochemical biosensor is also influenced by foulants that attach to the electrode surface via nonspecific absorption. The foulants can reduce the area of electrode surface that is available to the electrolytes, causing a decrease of the DPV signal. Prior to the detection of miRNA biomarkers in clinic samples, the performance of developed biosensor was first characterised in diluted serum. The selected miRNA biomarker solutions with concentrations of 0.1, 1, 10, 100, and 1000 pM were prepared in 2% fetal bovine serum (FBS), which was used as the foulant. It is remarkable that the control electrodes present extraordinary stability after incubated in 2% serum concentration for 20 minutes (Figure 7d), demonstrating the excellent antifouling property of developed sensing platform. As expected, successive current decreases were observed in the working electrodes with increasing miRNA titre in 2% FBS solutions (Figure 7e). The dosage response curve presented in Figure 7f provides a sensitivity of 0.03 pM^{-1} , which is slightly lower than the value in pure buffer solutions. The result indicates that the THCPsi biosensor can detect the miR-142-miRNA biomarker selectively down to a concentration of 0.1 pM in 2% serum.

Example 6 – Detection of target miRNA biomarkers in clinic samples

The capability of the optimised THCPsi nanochannel-based biosensors were further tested to detect miRNA biomarkers in clinic samples. Human serum samples collected from both healthy donors and mTBI patients were added into Tris buffer solution to obtain 2% final concentration. Figure 8a and b show the electrochemical responses of biosensors with complementary capture probe to miR-142-3p. The DPV current drop upon incubation in the positive samples for 20 minutes, compared to the identical signal in negative samples, demonstrates that the nanochannel-based biosensors are capable of effectively distinguishing human serum samples containing the specific miRNA biomarker from those not containing the biomarker. Another mTBI related miRNA biomarker, miR-423-3p, was also measured in the human serum samples, by simply changing the immobilised complementary ssDNA capture probe to miR-423-ssDNA-capture on the electrode surface. The sensing results, as shown in Figure 8c and d, further confirm the capability of developed miRNA biosensor to detect multiple

miRNA biomarkers related to TBI, allowing the accurate diagnosis of TBI within a 20 minutes time window.

According to the calibration curve obtained from 2% FBS buffer, the actual concentration of miR-142-3p in full human serum samples can be further determined by comparing the intensity ratios with the linear fit curve. Figure 9 clearly shows the calculated concentrations of miR-142-3p in three different clinic sample groups. These results were then compared with clinical PCR test results, which are summarised in Table 3. It is remarkable that the detection results from the electrochemical detection system are in excellent agreement with the PCR results obtained at the Alfred Hospital as well as the history of the TBI patients, which included a fall from a ladder, a traffic accident and a fall from scaffolding. Moreover, as the gold standard PCR technique can only determine the relative concentrations of miRNA biomarker in human serum samples, the electrochemical detection system is also able to further determine the accurate values of miRNA concentrations in the picomolar range within a 20 minutes window. The precise quantification of miRNA markers in human serum enables therefore allows an immediate assessment related to the severity of a TBI.

Table 3. Comparison between miRNA detection results obtained by PCR and the electrochemical detection system disclosed herein.

Sample ID	Clinical PCR (NER)	Exemplified System
Control 1	0.27	0
Control 2	0.359	0
Control 3	1.052	0
B20 (ladder fall)	0.938	5.35 ± 0.65 pM
B11 (traffic accident)	1.523	7.08 ± 0.71 pM
B9 (scaffolding fall)	4.060	15.46 ± 0.59 pM

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

CLAIMS

1. A method of detecting at least one nucleic acid analyte in a complex biological sample, the method comprising:
- 5 (i) introducing the sample to an electrochemical biosensor comprising a porous substrate comprising a plurality of channels, wherein:
- at least a portion of the plurality of channels has a mean depth of about 1.0 μm to about 6.0 μm ; and
 - at least a portion of the plurality of channels has a mean diameter or cross
- 10 section in a range of about 15 nm to about 40 nm,
- wherein one or more capture probes capable of binding specifically to at least biomarker, optionally at least one nucleic acid analyte, to form a probe-analyte complex, are disposed on at least one surface of at least a portion of the plurality of channels; and
- 15 (ii) determining the presence or absence of the at least one nucleic acid analyte in the sample based on measurements of peak current obtained from differential pulse voltammograms prior to and after introducing the sample to the electrochemical biosensor,
- wherein detection of a change in the peak current after introduction of the sample to the electrochemical biosensor indicates the presence of the at least one nucleic acid analyte
- 20 in the sample and detection of no change in the peak current after introduction of the sample to the electrochemical biosensor indicates that the at least one nucleic acid analyte is not present in the sample above a threshold level.
2. The method according to claim 1, wherein the capture probe is capable of binding specifically to at least one nucleic acid analyte to form a probe-analyte complex, wherein the presence of the probe-analyte complex within a channel causes at least a partial steric and/or charge blockage to the channel as compared to the channel in the absence of the probe-analyte complex.
- 25 3. The method according to any one of claims 1 to 3, wherein:
- at least 10% of the plurality of channels in the porous substrate have a mean depth in a range of about 1.0 μm to about 2.0 μm , or about 1.0 μm to about 4.0 μm ; and/or
 - at least 10% of the plurality of channels in the porous substrate have a mean
- 30 diameter or cross section in a range of about 15 nm to about 30 nm, or about 20 to about 30 nm.
- 35

4. The method according to any one of claims 1 to 3, wherein:
- the porous substrate comprises, consists essentially of, or consists of, a conducting material or semi-conducting material; and/or
 - 5 - the porous substrate comprises, consists essentially of, or consists of, a material selected from the group consisting of: platinum, gold, silver, cobalt, gallium, indium, tin, vanadium, zirconium, copper, aluminium, iridium, palladium, rhodium, silicon, zinc, iron, steel, brass, carbon, graphite, alloys, oxides, or metallic compounds of these elements, and mixtures thereof; and/or
 - 10 - the porous substrate is not in the form of a film; and/or
 - the porous substrate does not comprise nanoparticles and/or is composed of nanoparticles; and/or
 - the porous substrate does not comprise one or more of aluminium, platinum
 - 15 gold, and silver; and/or
 - the porous substrate comprises, consists essentially or consists of a material selected from silicon, optionally a heat treated and/or chemically modified silicon; and/or
 - a layer comprising, consisting essentially thereof, or consisting of, carbon
 - 20 is disposed on at least a portion of the porous substrate.
5. The method according to any one of claims 1 to 4, wherein:
- the porous substrate, optionally comprising silicon, comprises, or consists essentially of, or consists of, a single layer comprising one or more of:
 - 25
 - one or more capture probes capable of binding specifically to at least one biomarker, optionally at least one nucleic acid analyte, to form a probe-analyte complex; and/or
 - one or more functional groups capable of reacting with an amine group; and/or
 - 30
 - one or more functional groups capable of forming a covalent bond with a complementary functional groups on one or more capture probes that are capable of binding specifically to at least one biomarker, optionally at least one nucleic acid analyte, to form a probe-analyte complex; and/or
 - 35
 - one or more carboxylic acid groups; or

- the porous substrate, optionally comprising silicon, comprises, consists essentially of or consists essentially of or consists of at least one layer comprising one or more of:
 - 5 ▪ one or more capture probes capable of binding specifically to at least one biomarker, optionally at least one nucleic acid analyte, to form a probe-analyte complex; and/or
 - one or more functional groups capable of reacting with an amine group; and/or
 - 10 ▪ one or more functional groups capable of forming a covalent bond with a complementary functional groups on one or more capture probes that are capable of binding specifically to at least one biomarker, optionally at least one nucleic acid analyte, to form a probe-analyte complex; and/or
 - one or more carboxylic acid groups.
- 15 6. The method according to any one of claims 1 to 5, wherein:
 - the one or more capture probes are capable of binding at a plurality of different nucleic acid analytes; and/or
 - the at least one nucleic acid analyte is selected from the group consisting of: a DNA analyte, a messenger RNA (mRNA) analyte, ribosomal RNA (rRNA) analyte, a transfer RNA (tRNA) analyte, a microRNA (miRNA) analyte, a short interfering RNA (siRNA) analyte, plasmid DNA (pDNA) and mixtures thereof; and/or
 - 20 - one or more of the at least one capture probe is a DNA capture probe or a peptide nucleic acid probe (PNA) probe.
- 25 7. The method according to any one of claims 1 to 6, wherein:
 - the at least one nucleic acid analyte is associated with a condition, disease state or injury; and/or
 - 30 - the at least one nucleic acid analyte is associated with a brain injury, optionally a traumatic brain injury.
- 35 8. The method according to any one of claims 1 to 7, wherein the at least one nucleic acid analyte is a miRNA associated with a brain injury, optionally a traumatic brain injury.

9. The method according to claim 6 or claim 8, wherein the one or more miRNAs are selected from the group consisting of miR-142-3p, miR-196b-5p, let-7f-5p, miR-150-5p, or any combination thereof
- 5 10. The method according to any one of claims 1 to 9, wherein the at least one nucleic acid analytes is detected in the sample at a concentration of about 1 pM or less.
- 10 11. The method according to any one of claims 1 to 10, wherein the complex biological sample is or comprises a body fluid, cell culture medium, water sample, a soil sample, food or beverage sample, an agricultural product, surface debris, a surface swab or any combination thereof.
12. The method according to any one of claims 1 to 11, wherein the complex biological sample is:
- 15 - from a human; and/or
- a liquid sample; and/or
- selected from the group consisting of: whole blood, serum, plasma, urine, saliva, sweat, spinal fluid, sputum, cell lysate, cell culture medium and mixtures thereof; and/or
- 20 - obtained from a subject suspected of having suffered a brain injury, optionally a traumatic brain injury.
13. The method of any one of claims 1 to 12, wherein the electrochemical biosensor comprises one or more electrodes.
- 25 14. The method according to claim 13, wherein the electrochemical biosensor comprises:
- a working electrode comprising the porous substrate;
- a reference electrode; and
- 30 - a counter electrode.
15. The method according to claim 13 or claim 14, wherein an insulating material is disposed on at least a portion of the electrodes.

16. The method according to any one of claims 1 to 15, wherein the electrochemical biosensor is used in conjunction with (i) a device capable of scanning a potential within a certain range and measure current or (ii) a potentiostat.

5 17. The method according to any one of claims 1 to 16, wherein a change in the peak current is normalised via the relationship of Equation 1:

$$\Delta I = (I_0 - I_n)/I_0 \quad (1)$$

where ΔI is a normalised current change, I_0 is a peak current value measured after incubation for a first period of time in a buffer, and I_n is the peak current value measured
10 after incubation for a second period in a target solution at a given concentration, in comparison to a control sample.

18. The method according to claim 17, wherein the first period of time and/or the second period of time is independently about or less than about: 60 minutes, 50 minutes,
15 40 minutes, 30 minutes or 20 minutes.

19. The method according to any one of claims 1 to 18, comprising:

- determining the concentration of the nucleic acid analyte detected in the sample based on a level of reduction in peak current intensity;
- 20 - optionally comparing the concentration of the at least one nucleic acid analyte with a calibration curve; and
- optionally determining the severity of a disease state or injury, optionally a brain injury in a subject based on the concentration of the at least one nucleic acid analyte.

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20. The method according to any one of claims 1 to 19, wherein the at least one nucleic acid analyte is detected, and concentration optionally quantified, within: about 60 minutes, about 50 minutes, about 40 minutes, about 30 minutes, about 20 minutes, about 15 minutes or about 10 minutes, of introducing the sample to the electrochemical
30 biosensor.

21. A porous substrate when used in a method according to any one of claims 1 to 20, said porous substrate comprising a plurality of channels, wherein:

- at least a portion of the plurality of channels has a mean depth of about 1.0 μm to about 6.0 μm ; and/or
- 35

- at least a portion of the plurality of channels has a mean diameter or cross section in a range of about 15 nm to about 40 nm,
wherein one or more capture probes capable of binding specifically to at least one nucleic acid analyte to form a probe-analyte complex are disposed on at least one surface of at
5 least a portion of the plurality of channels.

22. The porous substrate when used according to claim 21, wherein the capture probe is capable of binding specifically to at least one nucleic acid analyte to form a probe-analyte complex, wherein the presence of the probe-analyte complex within a channel
10 causes at least a partial steric and/or charge blockage to the channel as compared to the channel in the absence of the probe-analyte complex.

23. The porous substrate when used according to claim 21 or claim 22 wherein:
- at least a portion of the plurality of channels has a mean depth of about 1.0
15 μm to about 6.0 μm ; and
 - at least a portion of the plurality of channels has a mean diameter or cross section in a range of about 15 nm to about 40 nm.

24. The porous substrate when used according to any one of claims 21 to 23, wherein:
- 20 - at least 10% of the plurality of channels have a mean depth in a range of about 1.0 μm to about 6.0 μm ; and/or
 - at least 10% of the plurality of channels have a mean diameter or cross section in a range of about 15 nm to about 40 nm.

25. The porous substrate when used according to any one of claims 21 to 24, wherein:
- the porous substrate comprises, consists essentially of, or consists of, a conducting or semi-conducting material; and/or
 - the porous substrate comprises, consists essentially of, or consists of, a material selected from the group consisting of: platinum, gold, silver,
30 cobalt, gallium, indium, tin, vanadium, zirconium, copper, aluminium, iridium, palladium, rhodium, silicon, zinc, iron, steel, brass, carbon, graphite, alloys, oxides, or metallic compounds of these elements, and mixtures thereof; and/or
 - the porous substrate comprises, consists essentially or consists of a material
35 selected from silicon; and/or

- a layer comprising, consisting essentially thereof, or consisting of carbon is disposed on at least a portion of the porous substrate.
26. The porous substrate when used according to any one of claims 21 to 25, wherein:
- 5 - the porous substrate, optionally comprising silicon, comprises, or consists essentially of, or consists of, a single layer comprising one or more of:
 - 10 ▪ one or more capture probes capable of binding specifically to at least one biomarker, optionally at least one nucleic acid analyte, to form a probe-analyte complex; and/or
 - 15 ▪ one or more functional groups capable of reacting with an amine group; and/or
 - 20 ▪ one or more functional groups capable of forming a covalent bond with a complementary functional groups on one or more capture probes that are capable of binding specifically to at least one biomarker, optionally at least one nucleic acid analyte, to form a probe-analyte complex; and/or
 - 25 ▪ one or more carboxylic acid groups; or
 - the porous substrate, optionally comprising silicon, comprises, consists essentially of or consists essentially of or consists of at least one layer comprising one or more of:
 - 30 ▪ one or more capture probes capable of binding specifically to at least one biomarker, optionally at least one nucleic acid analyte, to form a probe-analyte complex; and/or
 - 35 ▪ one or more functional groups capable of reacting with an amine group; and/or
 - one or more functional groups capable of forming a covalent bond with a complementary functional groups on one or more capture probes that are capable of binding specifically to at least one biomarker, optionally at least one nucleic acid analyte, to form a probe-analyte complex; and/or
 - one or more carboxylic acid groups.
27. The porous substrate when used according to any one of claims 21 to 26, wherein:
- the one or more capture probes are capable of binding at a plurality of different nucleic acid analytes; and/or

- the at least one nucleic acid analyte is selected from the group consisting of:
a DNA analyte, a messenger RNA (mRNA) analyte, ribosomal RNA (rRNA) analyte, a transfer RNA (tRNA) analyte, a microRNA (miRNA) analyte, a short interfering RNA (siRNA) analyte, plasmid DNA (pDNA) and mixtures thereof; and/or
 - one or more of the at least one capture probe is a DNA capture probe or a peptide nucleic acid probe (PNA) probe.
28. The porous when used substrate according to any one of claims 21 to 27, wherein:
- the at least one nucleic acid analyte is associated with a condition, disease state or injury; and/or
 - the at least one nucleic acid analyte is associated with a brain injury, optionally a traumatic brain injury.
29. The porous substrate when used according to any one of claims 21 to 28, wherein the at least one nucleic acid analyte is a miRNA associated with a brain injury, optionally a traumatic brain injury.
30. The porous substrate when used according to claim 29, wherein the one or more capture probes are capable of binding one or more miRNAs selected from the group consisting of miR-142-3p, miR-196b-5p, let-7f-5p, miR-150-5p and combinations thereof.